The application of dental microwear texture analysis to human remains from the Bronze Age archaeological site, Minferri. A methodological study into its potential and limitations.

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Abstract
Previous research has shown that dental microwear texture analysis (DMTA) is a key method in studying the diet of animals and humans in their last moments of life. Enamel surface textures are measured with parameters to quantify the microwear and be able to make dietary inferences about individuals and populations. The use of a confocal microscope and metrology software to analyse dental microwear signatures is a very recent development in this field of research. This has provided new perspectives but also presented challenges, issues and limitations that require addressing. For example, there is still a need for much experimentation and standardisation of parts of the method. Although many different species have been studied, this paper is one of the first in examining human buccal molar surfaces with DMTA. The objective of this research is not only to bring new information about the diet of prehistoric societies but also to reflect on the potential and limitations of the method and develop and apply a protocol to a sample of 7 individuals from Minferri, an early Bronze Age site in Catalunya, Spain. In order to explore the method further, 5 statistical tests with different variables were created and executed which have led to promising results. These results contribute to the ongoing effort of standardising the technique to improve the reliability, reproducibility and comparability when applied to archaeological human remains.

Resum
Investigacions prèvies han demostrat que l'anàlisi de la textura del microdesgast dental (DMTA) és un mètode clau per a l’estudi de la dieta dels animals i els humans en els seus últims moments de vida. Les textures de la superfície de l'esmalt es mesuren amb paràmetres per quantificar el microdesgast i poder fer inferències sobre l’alimentació d’individus i poblacions. L’ús d’un microscopi confocal i d’un programari de metrologia per analitzar el microdesgast dental és una metodologia que s’ha aplicat molt recentment en aquest camp d’investigació. Això obre noves perspectives però també planteja algun reptes, problemes i limitacions que cal afrontar. Per exemple, la necessitat de dur a terme més proves experimentals, i una major estandardització de la metodologia i dels paràmetres d’anàlisi. Tot i que s’han estudiat moltes espècies diferents, aquest treball de recerca és un dels primers que analitzarà superfícies vestibulars de molars humans a través de DMTA. Té per objectiu aportar noves dades sobre la dieta de les societats prehistòriques, i també reflexionar sobre les potencialitats i limitacions d’aquesta tècnica aplicada a dents arqueològiques, així com el desenvolupament i aplicació d’un protocol a una mostra de 7 individus humans del jaciment del Bronze Antic de Minferri (Juneda, Catalunya). Per aprofundir en el mètode, es van crear i executar 5 tests estadístics amb diferents variables que han aportat resultats prometedors. Els resultats d’aquest treball suposaran una nova contribució a l’esforç actual d’estandardització de la tècnica per millorar la fiabilitat, la reproductibilitat i la comparabilitat del les analisis DMTA aplicades a restes humans arqueològiques.

Keywords: Confocal microscope, Human dental microwear, DMTA, Paleodiet, Surface texture analysis, Iberian Peninsula

Paraules clau: Microscopi confocal, Microdesgast dental en humans, DMTA, Paleodieta, Anàlisi de textura en superfícies, Península ibèrica
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<th>Acronym</th>
<th>Full name</th>
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<th>male</th>
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<td>ante-mortem tooth loss</td>
<td>M_p</td>
<td>possible male</td>
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<td>ART</td>
<td>artificial resynthesis technology</td>
<td>MIN</td>
<td>Minferri</td>
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<tr>
<td>BCE</td>
<td>before common era</td>
<td>N</td>
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<td>Deoxyribonucleic acid</td>
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<td>enamel-dentine junction</td>
<td>SJ</td>
<td>sitja (silo)</td>
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<tr>
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<td>Sr</td>
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1. Introduction

Human dental remains are an invaluable source of anthropological information that provide us with a glimpse into the lives of past peoples. Not only can we learn about the demographics, health and diet of ancient populations, but also about the environments in which they lived. Moreover, dental remains can provide vital information on the evolution of different species and help draw comparisons between taxa as in the case of hominids, for example. Due to its high mineral content, enamel is the hardest substance in the human body and therefore most commonly preserved in the human fossil record. On the other hand, it is susceptible to degradation and, unlike bone, does not remodel itself during an individual’s lifetime thus enabling dental remains to be used as a record of the past. Macrowear and microwear investigations in humans have provided evidence of diet and tooth use. Phytoliths, abrasive substances in our diets, ways in which foods are processed and other non-ingesta related tooth uses, e.g. as tools or teeth-cleaning, all produce microwear patterns on enamel which can be analysed. The study of such has become known as dental microwear analysis (DMA) and more recently with the use of confocal microscopy and surface texture parameters, dental microwear texture analysis (DMTA). Despite being a fairly recent development, DMTA has been applied extensively on in vivo and fossilised teeth in both hominids and other toothed animals alike. However, little work has been carried out on modern human dental remains and as far is known, only one other investigation has yet been published using the confocal on human molar buccal surfaces (Hernando et al., 2022) which is the subject of this research work.

As in all scientific disciplines, DMTA comes with its problems and limitations that need to be understood in order to make accurate interpretations of the microwear patterns. Initially suggested as a solution to pre-existing issues in DMA, such as human observer error and instrument subjectivity, to date, there is still a call for standardisation, additional testing of
various aspects of the technique and the exchange and collaboration between research groups (Ungar et al., 2003; Weber et al., 2021; Winkler & Kubo, 2022). Recent technological advancements have enabled the results of microwear analysis to become more comparable and reproducible. However, the technology, having not originally been designed to use for tooth surfaces, requires further testing to fully understand the implications during the application to archaeological dental remains. Comparing results in dental microwear experiments, using specimens with known diets, has provided an understanding into how the array of wear patterns are produced. However, still large amounts of testing into the possible surface alterations and their causes remains to be carried out (Weber et al., 2022). Post-depositional surface modification (PDSM) stemming from taphonomy, for example, can further transform the tooth wear patterns and must be taken into consideration throughout the whole investigation. In this paper, by establishing a protocol for DMTA of buccal molar surfaces and carrying out preliminary testing, I explored the potential of DMTA for the analysis of Bronze Age human dental wear. Although the results obtained here have shown some interesting differences within the population of Minferri, they only provide a basis for wider-reaching, future investigations with larger sample sizes. This paper raises many questions and challenges for the future that still need to be addressed in more extensive studies.
2. Literature review

2.1 The development of dental microwear research

As early as 1933 an American palaeontologist, G.G. Simpson, published his PhD thesis on the different jaw movements in mammals and believed them to be related to the variety of foods they ate (Simpson, 1933). Early pioneers in the 1950s first started to make the connection between dental microwear patterns and dietary inferences (Butler, 1952; Mills 1955; Baker et al., 1959). However, it was not until the 1960s that the study of macro- and microwear features was applied to humans and dental anthropologists such as Dahlberg and Kinzey (1962) began to analyse the connection between tooth surface abrasion (wear from external objects), attrition (wear from the contact between upper and lower teeth) and the foods which had been consumed. Since then, other tribology studies have been carried out to distinguish these types of wear and also including the effects of erosion (wear mainly resulting from acids which affect the enamel) and abfraction (a loss of tooth structure near the gum line due to force placed on the tooth) (Grippo et al., 2004; Bartlett & Shah, 2006; Michael et al., 2009).

Work began into how an image of the tooth’s surface could be translated into quantitative data and the focus was placed on pits and striations that were manually measured, orientated and analysed. Indeed, tooth wear indices have allowed archaeologists to quantify the varying degrees of “macrowear” (Eccles, 1979; Smith & Knight, 1984), a term introduced to anthropology by E. Scott in 1979 to describe dental tissue loss on the occlusal surface (Scott, 1979) later the term “mesowear” was applied to the study (Fortelius & Solounias, 2000). Most indices are numerical and quantitative, however, partly due to the large variety, none of which having gained universal acceptance, there is still a need for further research and standardisation. (Bardsley, 2008; Shykoluk & Lovell, 2010; López-Frías et al., 2012). As early as the 80s, in DMA Gordon and Teaford amongst others recognised the need for more standardised, objective
protocols and further automation to process the enormous amounts of data that even small samples produce (Gordon, 1987; Teaford, 1988).

Initial investigations into dental microwear using optical light microscopy (OM), however, came with significant limitations. Not only the difficulty of analysing curved surfaces of teeth, but also insufficient magnification and resolving power limited the imagery. Since the late seventies, the scanning electron microscope (SEM) has been used as it allows smaller microwear features to be seen. Despite what have been called “seductively realistic images” from the SEM (Ungar et al., 2003), issues with using the technique for DMA soon became apparent. Apart from the loss of data resulting from the reduction of 3D surfaces to 2D representations, the images are also dependent on the instrument settings and orientation of the specimen e.g. the voltage, working distance or surface tilt. The analysis process is time-consuming, costly and can have high observer error rates, from between 9% to 19% on average (Grine et al., 2002). These problems led a growing number of scientists to favour low-magnification light microscopy, which they believed to be more effective (Solounias & Semprebon, 2002; Semprebon et al., 2004; Merceron et al., 2004). Although the technique is simpler, quicker and cheaper, it still did not address the limitations of high observer error rates and reproducibility. However, low-magnification dental microwear analysis (LMDA) continues to be applied successfully and it was proven that the observer error is reduced in more experienced users (De Santis, et al., 2013).

For the first time in 1991, Boyde and Fortelius published recommendations on the use of confocal microscopy in dental microwear analysis (Boyde & Fortelius, 1991) and now for many, it has become the microscope of choice for enamel surface texture analysis. The confocal has been previously used in a wide variety of experiments ranging from molecular imaging in cancer diagnosis (Kiesslich et al., 2007; Fuchs et al., 2013; Ahlgrimm-Siess et al., 2018) to traceology analysis of archaeological and experimental lithic tools (Evans & Donahue, 2008;
Stemp et al., 2015; Ibáñez & Mazzucco, 2021). It has been argued that the fields of dental microwear and traceology should collaborate thereby learning from each other’s experience and experimentation (Calandra et al., 2019a). Bones have also been analysed in experiments using confocal microscopy and multivariate analyses. In one case, use-wear traces were measured on unworked bone and compared with archaeological bone tools with the aim of better understanding the texture patterns created (Martisius et al., 2018).

For almost 20 years, the confocal has been coupled with scale-sensitive fractal analysis (SSFA; Ungar et al., 2003) as a tool to analyse the 3D scans of tooth surfaces and this has become known as dental microwear texture analysis (DMTA). This type of analysis has been proven to be less arbitrary than other types of DMA, and therefore reproducible, replicable and potentially comparable. Pits and scratches are no longer counted manually and instead, the surface texture is characterised using several parameters whilst also taking into account the scale of the observation area. The whole process is less time-consuming and costly than the SEM, once the initial investment in the confocal microscope and necessary software has been made. Ungar and team proposed DMTA as a solution to the previously mentioned limitations, in particular the lack of standardisation and the prevalence of observer error. Now using the scale-sensitive fractal parameters (SSFA) parameters, measurements could be made that are automated and repeatable (Ungar et al., 2003).

Figure 1 – Diagrammatic representation of tooth wear surfaces with different levels of complexity and anisotropy (source: Ungar et al., 2007)
The main SSFA parameters that have been used in DMTA are as follows: area-scale fractal complexity ($Asfc$), heterogeneity of complexity ($HAsfc$), scale of maximum complexity ($Smc$), exact proportion Length-scale anisotropy of relief ($epLsar$) and texture fill volume ($Tfv$). The complexity parameters refer to the variation of surface features i.e. roughness, whereas anisotropy refers to the direction and orientation concentration of the surface features (Figure 1). High complexity values mean a higher level of surface roughness and relief, on the other hand, high anisotropy values mean more uniformity in the wear patterns on the tooth. $Tfv$, refers to the amount of surface removed due to the tooth wear features (Schmidt et al., 2015). It has been suggested that $epLsar$ and $Tfv$ are the most discriminant parameters in this type of analysis (Krueger, 2015), but $Asfc$ and $epLsar$ tend to be the most frequently for discerning differences in diet among various groups (Scott et al., 2006; Ungar et al., 2012; Arman et al., 2016; Mahoney et al., 2016; Schmidt et al., 2019). ISO 25178 surface roughness parameters (found in the international reference collection: Geometrical Product Specification (GPS) - Surface texture: areal\(^1\)), used in a wide range of surface texture analyses (STA), have also proven to be effective in the analysis and characterisation of dental microwear (e.g. Schulz et al., 2013; Calandra et al., 2016a; Mihlbachler et al., 2019). Throughout this study all possible parameters will be considered to measure the surface topography patterns\(^2\).

Surfaces with low $epLsar$ are more isotropic as they have less variation in the lengths and orientation of the features. With regards to dietary interpretations, this has been related to taxa that consume softer, tougher items which cause a more striated surface (Scott et al., 2005, 2006; Ungar et al., 2012). The anisotropy parameters have also often been attributed to phytoliths and fibrous diets, including tubers and grasses, for example. (Krueger & Ungar, 2010; El-Zaatari, 2010; Schmidt et al., 2015, 2019). In contrast, species that consume more

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\(^2\) See Table 5 for detailed descriptions of all the parameters used in this study.
hard-brittle foods tend to have higher $Asfc$, or complexity, which reflects change in roughness and features such as pits; A surface dominated by features of varying sizes superimposed over one another would have a high $Asfc$ value (Ungar et al., 2012). However, these results all come from occlusal surface research which has been shown to have contrasting parameter values, in particular, lower $epLsar$ values than buccal surfaces (Hernando et al., 2022).

Higher anisotropy values can also be a result of attrition wear, for example, and high complexity levels due to corrosive wear from dietary acids (Hara et al., 2016; Ranjitkar et al., 2017). Meat, however, has shown to leave little or no microwear, except where it contains additional, exogenous dust and grit that can easily transform the texture patterns (Teaford & Lytle, 1996; El-Zaatari, 2010; Hoffman et al., 2015; Merceron et al., 2016; Ackermans et al., 2020; Teaford et al., 2021). Therefore, the manner in which food is processed, e.g. milled cereals and cooking practices, can change microwear and this needs to be taken into account when interpreting results. In order to study and compare dental microwear patterns in human populations, dietary variations and subsistence strategies have been categorised into the following groups: hunters-gatherers, fish-eaters, farmers, foragers, pastoralists and agriculturalists (e.g. Jarošová et al., 2006; Schmidt et al., 2016, 2019; Williams et al., 2018). Farmers, pastoralists and agriculturalists generally have shown to have higher anisotropy parameter values and foragers lower results (Schmidt et al., 2019). Furthermore, a study carried out on human dental remains from the Late Bronze-Early Iron Age transition showed an increase in microwear complexity thought to be due to an increased reliance on agricultural goods and reduced dependence on ovicaprines (Van Sessen, et al., 2013).

Additionally, it is important to bear in mind that although we can learn about diets, it is only a record of the individual’s last weeks or months of their lives. This has become known as “the Last Supper” effect (Grine, 1986) and is particularly important to consider with species whose diets change seasonally (Merceron et al., 2010, 2021). Experimentation with rats given
food with different levels of abrasiveness has shown that microwear patterns, on average, were renewed within 14 to 26 days. Whilst changes in the enamel were seen from day two, it took at least two weeks for texture patterns to start overwriting themselves (Winkler et al., 2020). Less abrasive diets tend to generate more anisotropic surfaces i.e. less uniform and also overwrite and erase the features below in a slower manner than more abrasive foods (Schulz et al., 2013).

More recently, attempts have been made to standardise the methodology, however, hitherto no procedure has been agreed upon. Standardisation would allow for knowledge and data to be shared to better understand DMTA and increase future inter-lab collaborations. It has been demonstrated that the dataset can be influenced by the format in which the confocal scans are saved, filter types, patching techniques and by the type and age of the microscope used. Amongst others, Arman et al. (2016) have proposed a pre-analysis treatment protocol to minimise inter-microscope variability in an ongoing effort to make research more comparable. Kubo et al. (2017) carried out a study to test this using 2 confocal microscopes at 2 magnifications and 3 different types of pre-analysis templates which were applied to the scans. They found that the application of different filters, which are part of the template, had a greater effect on the results than the model of confocal. Thereafter, Winkler and Kubo joined forces to carry out investigations into inter-microscope and filter variability and provided a recommendation on which parameters to use (Winkler & Kubo, 2022 preprint). As yet, no conclusions have been made on which pre-analysis protocol should be used in the future due to the fact that more comparative studies are required.

Furthermore, microwear features may lose a degree of relief and angularity due to the viscosity of moulding and casting materials. Therefore, different types of silicon-based impression media have been tested for precision by comparing scans of the mould to the same area on the tooth surface. The results show that low and mid-viscosity materials, in particular President Jet Light and Regular Body (Coltène Whaledent), are recommended in order to
produce comparable negative moulds (Galbany et al., 2004; Goodall et al., 2015). Positive casts were compared with the original tooth surface and found to have significant differences between most of the ISO parameter (i.e. 26/34) values (Mihlbacher et al., 2019).

Many other aspects can also vary within a study. A variety of microscope magnifications have been applied, 100x being the most common. Human anterior teeth have been analysed (Krueger and Ungar, 2010; Mahoney et al., 2016; Krueger et al., 2019), however, predominantly molars are used, in particular the second molar. Fossil hominin occlusal microwear signatures were compared using SSFA parameters and no significant difference between the first (M1) and second (M2) molars was found (El-Zaatari, 2010). Very few comparison studies between different molars have been carried out and to increase sample sizes M1s and M2s are normally combined. Different tooth surfaces i.e. buccal or occlusal have also been analysed, however, in DMTA the occlusal surface is by far the most frequently used. Specific facets on the occlusal surface have also been examined, facet 9 and 11 being the most studied Phase II grinding facet (Figure 2). Comparisons between Phase I (1-8) and Phase II (9-13) facets in the masticatory cycle have been made. Phase I facets are also known as shearing surfaces and Phase II facets are involved in the grinding and crushing actions. Ramdarshan et al. (2016), although studying ewes (Ovis aries), demonstrated how including different types of occlusal facets and molars from the upper and lower mandibles in one study can have an effect on the results. On the whole, further research is needed, however, microwear patterns on Phase II facets in primates have proven to better distinguish diet than Phase I facets (Krueger et al., 2008). Intra- and inter-facet texture variations in deciduous teeth from extant and mediaeval children have also been studied. No significant differences between facets 9 and 11, nor between large and small facet surface areas were found (Bas et al., 2020).
To date, many species from animals as small as voles (*Microtus agrestis*) (Calandra et al., 2016 a/b) to large dinosaurs (*Jinyunpelta sinensis*) (Kubo et al., 2021) have been the subject of confocal analysis. A variety of hominin teeth have been studied including comparisons between Neanderthals and early modern humans (Krueger et al., 2019) as well as the earliest hominins, *Australopithecus anamensis* and *Australopithecus afarensis* (Ungar et al., 2010). Schmidt and colleagues applied DMTA to Homo sapiens and found that the diets of foragers, farmers, and pastoralists could also be distinguished (Schmidt et al., 2015, 2019). In the same way, extant and mediaeval children’s deciduous occlusal surfaces have been analysed (Mahoney et al., 2016; Bas et al., 2020). So far, very few DMTA investigations have been made on modern adult humans and the studies that exist have focused mainly on the occlusal surface (El Zaatari, 2010; Schmidt et al., 2015, 2019; Pérez-Pérez et al., 2018; Hernando et al., 2022).

Experimental archaeology has been carried out to better understand the wear resulting from different food types (Merceron et al., 2016; Hoffman et al., 2015; Karme et al., 2016; Ramdarshan et al., 2016). However, little experimentation has been conducted on *in vivo* humans and the developments so far demonstrate the complications that are involved and the necessity for this type of investigation (e.g. Romero et al., 2012, 2013; Correia et al., 2021). With regard to human diets, there can be very subtle variations and combinations of food items.

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**Figure 2** - First molar (M1) occlusal surface with phase I (blue and green) and II facets (red) (source: Benazzi et al., 2011)
and little is known about which foods and/or processing techniques produce exactly which wear signatures. *In vivo* experiments have even shown that oral biofilm (the dental pellicle and bacterial layer) affects the sampling, mould precision and therefore the results (Correia *et al.*, 2021).

Experimenting with and studying taphonomic processes has advanced our knowledge of how enamel can easily be transformed over time and how to interpret this under the microscope (King *et al.*, 1999; Aliaga-Martínez, 2015; Uzunidis *et al.*, 2021). These studies have shown that ante- and post-mortem dental wear can be distinguished, however, to improve identification and diagnostic skills of inexperienced users initial training from professional researchers is essential (Willman *et al.*, 2020; Weber *et al.*, 2021). Avoiding the cusp tips and edges of teeth is recommended as they are most likely to suffer from taphonomic alterations (Uzunidis *et al.*, 2021). Taphonomic simulation experimentations that have been carried out with acid/alkali solutions or different sized sediments provide more information in order to have a larger overview of the cause of post-mortem damage and identify it more easily. Results from previously published experimentations have shown that in general abrasives can contaminate enamel surfaces but tend not to completely overwrite the ante-mortem texture patterns and can be distinguished. (Teaford, 1988; King *et al.*, 1999; Martínez & Pérez-Pérez, 2004; Weber *et al.*, 2022). Parameter values, in particular measuring height and volume variation, have been proven to change in tooth surfaces from rabbits with more abrasive diets (Martin *et al.*, 2020). Although many of these published works have been carried out with SEM and mainly on occlusal surfaces, it is still clear that abrasives such as grit and sand have an effect on the enamel and silica bodies such as phytoliths also produce alterations to the texture (Guegel *et al.*, 2001; Aliaga Martinez, 2015; Böhm *et al.*, 2019).

Visual reference guides, such as by Weber *et al.*, documenting confocal scans of post-mortem altered enamel surfaces are very useful tools to enlarge our knowledge and ability to
identify anomalies (Weber et al., 2021, sup. material). Inter-disciplinary investigations, for example, dental calculus analyses (Power et al., 2015; Bucchi et al., 2019) could bring a wider perspective and an enriched knowledge with regards to dietary inference. Stable carbon and nitrogen (δ13C and δ15N) isotope analyses have added another facet to our understanding of past diet variations as a longer indicator of diet and have successfully been used in combination with DMA to reconstruct paleo diet patterns (El-Zaatari, 2010; Grine et al., 2012; Salazar-García et al., 2016; Pérez-Pérez et al., 2018; Hernando et al., 2021). The confocal has also been combined with Artificial resynthesis technology (ART 5), a simulator that recreates the chewing cycle (Kreuger et al., 2021) and also 3D modelling of microwear in molars to create VR representations that help determine the effects of masticatory movements (Tausch et al., 2015).

As can be seen from the above, there is a great versatility to DMTA and even though still in its infancy, there is a huge potential for this technique to be used in dietary inference despite its remaining important issues. Amongst some of the many problems DMTA faces is the reduced sample sizes due to the condition of the enamel and post-mortem alterations to the dental remains (Ungar et al, 2006, 2008; Teaford, 2007; Krueger, 2015; Pérez-Pérez et al, 2018; Hernando, et al., 2020, 2021; Correia, et al., 2021). Not only can the enamel be transformed by pathologies but also by calculus and/or perikymata, external incremental growth lines. The alterations that the tooth can undergo are numerous both ante-mortem and taphonomically. Studying microwear in humans adds further complications due to the variety of human behaviours, from food preparation techniques to using teeth as tools or dental hygiene practices. Furthermore, distinguishing the subtleties between subsistence strategies and diets can be challenging because people’s customs, geographically and over time, tend to overlap (Schmidt et al., 2019). In the future, standardisation of DMTA techniques and protocols would be required in order to allow data to be combined and compared in meta-analyses. Mould
preparation, pre-analysis treatments and data analysis processes all have proven to have an impact on the results which needs to be fully investigated and understood. Further analyses and controlled food experimentations are required to increase our understanding of the microwear patterns left on enamel. In this paper, I endeavour to investigate some of these issues to better understand the potential and limitations of confocal microscopy and modern human DMTA.

2.2 Minferri - Archaeological site description

The individuals under investigation all come from an open-air archaeological site in the North-East of the Iberian Peninsula (Figure 3). Minferri is located on the western Catalan plain near the village of Juneda in the province of Lleida. Situated inland, the area has a continental Mediterranean climate and very fertile land with access to plentiful natural resources (Figure 4).

Figure 3 - Aerial photo of Minferri (south-north view) (source: Nieto-Espinet et al., 2014)
There were two main occupational phases during the late Neolithic and the Bronze Age. A total of 18 radiocarbon dates were taken from various samples of human and fauna bones, a seed and charcoal. The results demonstrated that the main occupational phase was from 2100 – 1650 cal BCE (Figure 5), which corresponds to the Bronze Ple period (Equip Minferri 1997; Alonso & López, 2000; López, 2000, 2001). Minferri’s occupational phases have been established not only on the basis of the regional periodisation of the western Catalan plain derived from the C14 dating series. Other aspects such as the typology of the habitat and urbanism, and the evolution of economic strategies, social organisation and funerary practices have also been taken into account. In any case, as with the Ancient and Middle Bronze Age periods (Bronze Inicial), the Bronze Ple would be grouped under the terminology of Early Bronze Age used in other areas of the Catalan territory (Figure 6).
Figure 5 - Published radiocarbon dates from Minferri (source: Nieto-Espinet et al., 2014)

Figure 6 - Periodisation with calibrated dates of the western Catalan plain and surrounding regions (source: López, 2000)
Within the archaeological remains, post holes show that the hamlet was made up of a series of round huts constructed with perishable materials such as wood and adobe (Alonso & López, 2000). A significant discovery was the variety of different shaped pits ranging from open types e.g. cylindrical and closed types e.g. biconcave (Prats, 2013). They were mainly used for storing cereals and some were, subsequently, reused as rubbish pits or funerary structures for the individuals in discussion here. There were also production areas within the settlement that contained the remains of fireplaces and significant evidence of metallurgy activities including mineral reduction and bronze recasting. However, very few metal artefacts were unearthed on site and since remains were only found in a small number of concentrated areas, it has been hypothesised that the process was undertaken by a few specialised craftspeople with a considerable level of mastery (Equip Minferri, 1997; López & Moya, n.d.; Rovira i Hortalà, 2006).

The main economic activities were agriculture-based, largely dedicated to cereal farming and livestock breeding. From the archaeological record, a predominance of naked wheat (Triticum aestivum/durum) can be found, as well as hulled barley (Hordeum vulgare), flax (Linum usitatissimum) and evidence of harvested wild fruits and nuts such as blackberries (Rubus fruticosus) and acorns (Quercus sp). From a total of more than 5,100 organic remains samples, 49 different taxa of wild and cultivated plants from the archaeological excavations have been identified (Alonso, 1999). One plant of note, in particular for this study, is the mastic tree (Pistacia lentiscus). This plant is still used in parts of the world today for chewing gum, cooking and medicinal purposes due to its antibacterial and antifungal properties. On site, no evidence of the consumption of pulses was found (Alonso, 1999) which is fairly usual for the geographical location of the site.
Livestock remains found at Minferri included those of sheep (*Ovis aries*), goats (*Capra hircus*), cows (*Bos taurus*), horses (*Equus caballus*) and pigs (*Sus domesticus*) along with evidence of wild animal hunting, i.e. remains of deer (*Cervidae*) and hare (*Lepus europaeus*) were also discovered. Sheep and goats were the most prevalent animal remains found and, unusually, dogs (*Canis lupus familiaris*) and foxes (*Vulpes vulpes*) had also been deposited in silos often accompanying human burials (GIP, 2001). From the analysis of cattle (*Bos taurus*) remains (aged at 4+), suggestions have been made that the animal was not kept for meat but for physical labour such as ploughing (López, 2000). On the other hand, the majority of pigs that had been slaughtered were young, presumably, to take advantage of the meat as it was an important part of the inhabitants’ protein intake (Gómez, 2000). Faunal remains were discarded in waste pits. However, many animals and disarticulated body parts had been left as offerings either buried with humans or in separate silos (Nieto-Espinet *et al.*, 2014; Grandal-d’Anglade *et al.*, 2019). It is of note that very few bones were found with traces of burning, unlike the many pots analysed and, therefore, it is believed that the meat was boiled and not roasted directly on the fire (GIP, 2001).

Lithic tools were uncovered in significant numbers ranging from large saddle quern stones to microlithic flint blades and flakes. From lithic use-wear analysis evidence of meat cutting, animal hides, wood, cereals and mineral processing have been discovered (Marín Castro *et al.*, 2017). However, the most common finds on site were pottery fragments which varied in size and shape, some of which were decorated. The large variety uncovered, which has been classified by archaeologists, correspond to standard typologies from the period and geographical area as well as rarer items, such as fragments of cheese strainers and large decorated storage vessels (Equip Minferri, 1997). Other items were also recovered during the excavations such as shell jewellery, beads and bone needles (Alonso & López, 2000).
On the whole, the people of Minferri lived a sedentary life nevertheless in order to source raw materials, i.e. flint, or livestock pastures they would have ventured into the surrounding countryside (Equip Minferri, 1997). The population lived in close contact with nature and a large variety of fauna and flora. Animals were paramount for the society as part of their diet, for rituals, raw materials, companionship and carrying out physical labour. Not only were animals bred and kept for meat, but also secondary products such as milk. Their diet was varied including all kinds of domesticated, cultivated and wild foods mixing proteins and carbohydrates amongst others (Grandal-d’Anglade et al., 2019). The diverse variety of animal and plant remains, storage pits, ceramic vessels, food processing and agricultural tools suggest a high level of self-sufficiency in terms of subsistence strategies. The large quantity of quern stones, made of granite or other abrasive stones from the area, indicate that flour was an important part of their diet (GIP, 2001). The high capacities of the silos suggest there being a surplus of the harvest providing reserves for the following year, a certain degree of commensality and perhaps trade (López, 2001; Albizuri et. al., 2011; Prats, 2013). These sizable communal silos (e.g. 3,000-5,000 litres) point to a need for the management and redistribution of their contents which probably meant some kind of hierarchical leadership (Prats, 2013). Beside agricultural work and hunting, the people also carried out crafts such as metallurgy, sewing, pottery, carpentry and jewellery and tool making. Even after death, the inhabitants of Minferri stayed within the community, buried in repurposed silos dispersed amongst the hamlet and as far as is known, not in a separate necropolis.

2.3 Archaeological investigations at Minferri

The site was discovered in 1980 and the first intervention was carried out by Professor Joan Maluquer de Motes and his team in 1981. The Grup d’Investigació Prehistòrica (GIP) at the University of Lleida then ran 8 campaigns from 1993 until 2006. These were mainly rescue
excavations due to the construction of the Spanish high-speed railway line and since then no further work has been undertaken. To date, approximately 2.5 out of a probable 10 hectares of the site has been excavated and 425 structures have been examined (Figure 7 & 8). In 24 silo-shaped structures anthropological remains have been found, corresponding to a minimum of 56 individuals (Nieto-Espinet et al., 2014) which were studied by the anthropologists, Bibiana Agustí Farjas and Dolors Codina Reina (Agustí et al., 2003; Agustí, 2007, 2009). Each stratigraphic unit (unidad estratigráfica, UE) and structure was given a unique identification number and initials (by way of example for the silos or pits ES-296 or SJ-400 (Equip Minferri, 1997).
Other investigations such as DNA and stable isotope analyses have been undertaken. Genetic analyses were carried out on 16 individuals from two collective burial structures (SJ-399 and SJ-418). Results were obtained for 9 individuals, with a total of 545 mtDNA sequences. These analyses were conducted by the Forensic and Population Genetics Group (Department of Legal Medicine, Psychiatry and Pathology) at the Faculty of Medicine in the University Complutense, Madrid. The results of the study are still pending publication. In addition, extensive isotopic analyses have been applied to characterise the diet of humans and animals at the site (Grandal-d’Anglade et al., 2019), as well as to study the mobility and geographical origins of the individuals (Nieto-Espinet et al., study under publication).
Both stable isotope and dental microwear studies are commonly used methods for past human dietary reconstructions. The application of these analytical techniques in Mediterranean Iberia has significantly increased during the past few years. One such study is currently being undertaken for Minferri. With regards to diet from the study of $\delta^{13}C$ and $\delta^{15}N$ isotopes, the results of the analysis seem to show that the female individuals generated a wider distribution of both carbon and nitrogen values than males which suggests a greater variety in feeding practices. Also, some females had equivalent or even higher $\delta^{15}N$ values than males which is unusual as it suggests a larger intake of protein in their diets. The relatively low values in the $\delta^{13}C$ results also suggest that the people of Minferri did not consume large amounts of animal proteins on a regular basis. Curiously, some of the dogs and foxes found buried in the same structures as females and youths had similar isotopic values. This suggests possibly similar diets, a high level of interaction between the two groups and perhaps also a certain amount of nurturing from the humans (Grandal-d’Anglade et al., 2019).

More recently it has been possible to carry out a very broad sampling for the study of mobility dynamics for the inhabitants of Minferri. This is the first study on human mobility during late prehistory in Catalonia. Strontium isotopes ($^{87}\text{Sr}/^{86}\text{Sr}$) from human dental enamel were used to identify the presence of non-local individuals in the group. Samples were taken from 12 structures and 20 inhumations composed of men and women from the Bronze Age period (2100-1600 cal. BCE). The results suggest that the mobility diversity does not seem to be associated with the different diet types identified nor the sex of the individuals. This study, currently being published, has been led by Ariadna Nieto-Espinet, Natàlia Alonso y Andreu Moya from the Prehistoric Research Group of the University of Lleida, with the collaboration of Silvia Valenzuela-Lamas (IMF-CSIC) and Leopoldo Pena (GRC Geociències Marines, Faculty of Earth Sciences, UB).
2.4 Funerary structures and practices

*Minferri* has the largest funerary record as well as the first documented examples of reusing storage silos as graves in the western Catalan plain for the Early Bronze Age (Alonso & López, 2000). There were two concentrations of funerary pits and some were dispersed between domestic structures within the hamlet. From all the burials uncovered, 63% were in sector 2, zone 2 where 32 individuals were found in 10 different structures (Figure 9). The second largest concentration was in zone 9 where 6 individuals were buried in 5 distinct structures (Grandal-d’Anglade *et al*., 2019).

A wide diversity of funerary practices and burial patterns have been identified. There is also a lack of consistency with regards to the positions in which the individuals were laid out i.e. prone, supine or crouched (Figure 10). The majority were buried in single graves, however, double and collective pits with up to 11 individuals were also used. The 11 individuals in silo 418 (SJ-418) were buried separately over a period of time, except for one simultaneous quadruple burial with a pregnant woman and 2 infants (Agustí, 2009).
Figure 9. General plan of Minferri and location of the structures with all human burials, the total number of individuals sampled and the number of samples finally selected for DMTA. (source: GIP, UdL, 2022)
Figure 10 - Collection of photos of the different typologies of burials at Minferri: 1. SJ-354 (burial EN-467)(MIN-354-9085); 2. SJ-399 (burial EN-426)(MIN-399-5274); 3. SJ-399 (burial EN-411); 4. SJ-418 (burial EN-448)(MIN-354-5312); 5. SJ-399 (burial EN-420)(MIN-399-5252); 6. SJ-399 (burial EN-414)(MIN-399-5220); 7. SJ-95 (burial EN-135)(MIN-95-7086); 8. SJ-355 (deposition of two female femurs and a fox UE 9078). (Source: Nieto et al., 2014, fig.4) (bold: sample used for DMTA)
During this period at Minferri the treatment of the dead has been understood as ritualistic and comparable to other similar sites in Catalonia such as Can Roqueta and Mas d’en Boixos (Rodríguez et al., 2003; Bouso et al., 2004; Carlús Martín, 2021). This hypothesis is based on the quantity of grave goods which included ceramic items, silex and mainly intentional animal deposits such as cows, sheep, goats, dogs and foxes which were deposited whole, or selected parts with anatomical connection. Intriguingly, no pig remains were found along with the human burials (Grandal-d’Anglade et al., 2019). It has been suggested that these offerings were carefully selected with the intention to offer either protection and accompaniment into the next life and/or as a status identifier (GIP, 2001). Furthermore, in Minferri some separate non-funerary pits that only contained animal deposits were found less than 10 metres from the human burials (Prats, 2013). Even though there are some differences between the grave goods, there is very little evidence to suggest any kind of hierarchy other than a chiefdom amongst the inhabitants of Minferri (López, 2000, 2001; GIP, 2001; Nieto-Espinet et al., 2014). This is very unlike the Argaric cultures that were mainly concentrated in the south-east of the Iberian Peninsula around the same period and appeared to have had much more asymmetry in the social structure as evidenced by the grave goods (Lozano et al., 2021).

Out of all the individuals found at Minferri only two had exceptional variety and quantity of grave goods in the pit structure, SJ-88. The male skeleton was unusually old for the Bronze Age, estimated to have died between 60-70 years old (Figure 11). The female, aged 20-35, was buried at a lower strata and, therefore, earlier in date (Figure 12). Laid beneath the woman and above a possible cover or marker stone slab, was a deer horn, a goat without legs and two foxes. At a slightly higher level a new-born was buried along with a piece of flint. The elderly male in a crouched position with a north-south orientation had been placed to one side. He had been buried with the thorax of an ox, as well as the limbs of at least 7 goats (Agustí et
al., 2005; Nieto-Espinet et al., 2014; Grandal-d’Anglade et al., 2019). All these burials suggest that a set of complex funerary rituals took place.
Figure 11 - Photos of mandible from male (known as Big Man) excavated from silo 88 (MIN-88-2145)

Figure 12 - Photos of mandible from female found below Big Man in silo 88 (MIN-88-2183)
3. Objectives

The main aim here is to establish a protocol for the study of human molars using the confocal microscope and DMTA. To achieve this, it is fundamental to explore the potential and limitations of the technique when making dietary inferences from traces on dental remains. The case study, Minferri, will be used to better understand and illustrate the complexity in reconstructing diets from microwear in modern humans. Through critical reflection of existing studies, a methodological approach will be taken and applied to exploratory experiments into this relatively new technique. As has been demonstrated by previous works, many aspects can vary within an analysis of this kind and should be taken into account. As part of this study, questions will be asked such as: are there differences in microwear between sexes or between age group? Between the first and second molars? Do the results from the original tooth surface and the moulds differ a great deal? A preliminary assessment of the variability between the different molars and their diagnostic zones will be carried out, as well as contrasting microscopic scales of observation (100x and 200x). Through the use of multivariate statistical analysis, the individuals will be compared to determine whether groups in the sample differed in microwear texture patterns. Moulds will also be compared with the tooth surface to test the hypothesis that the third mould is the most precise replica.

One ongoing aim for researchers of DMTA is to introduce more standardisation in some aspects of the technique and determine the intra-/inter-observer variability in an attempt to increase reproducibility, repeatability and therefore, comparability. This study will contribute towards these efforts primarily by providing a clear explanation of the protocol and method applied to allow the dataset to be used for future investigations. This study will serve as an introductory analysis of DMTA and a step towards further, more extensive and comparable research work.
4. Materials

4.1 The anthropological ensemble from the Minferri site

For this study, the collection of mandibles was provided by the members of the *Grup d’Investigació Prehistòrica* at the University of Lleida (GIP) and studied using the confocal microscope at the laboratory of Social Dynamics Group of the *Milà i Fontanals* Institution (IMF) of the Spanish National Research Council (CSIC) in Barcelona. Out of the total 56 individuals (Table 1), 27 mandibles were made available to study under the confocal. The other 29 were either young individuals (<18 years old) (*n*=23) or missing the mandible (*n*=6). From the 56, there were 11 females (F), 7 of them determined as possible (F_p), and 8 males (M), 6 of them as possible (M_p). Lastly, 24 of the mandibles corresponded to individuals that could not be determined at the sex level (nd) due to deterioration of the remains or age of the individual. Regarding the age groups represented there were 16 mature individuals (6 F, 3 F_p, 4 M, and 2 M_p), 14 adults (5 F, 3 F_p, 3 M, 2 M_p and 1 nd), 4 adolescents aged 12-18 years old (1 F, 1 F_p and 2 nd), 18 children (2-12 yrs) and 4 infants (< 2 yrs) (Figure 13). Noteworthily, it transpires that 45 out of the 56 total samples were buried in collective burials of 2, 3, 5, 6, 8 and 11, and the rest individually.

![Graph](image)

Figure 13 - Graphs of all 56 individuals found at Minferri categorised by sex and age group
In order to identify the samples for this study, each individual was given an ID number consisting of MIN for Minferri, the pit structure where the remains were found and the related stratigraphic unit number e.g. MIN-399-5220. The initial sample of 27 individuals (Table 2) was composed of 9 F, 5 F_p, 8 M, 3 M_p and lastly, 2 nd. Regarding the age groups represented there are 15 mature individuals (6 F, 3 F_p, 4 M, and 2 M_p), 9 adults (3 F, 1 F_p, 4 M, and 1 M_p), 2 adolescents (1 F_p and 1 nd) and one child (Figure 14).

They were all excavated from reused storage pits that were a maximum of 3 metres deep with a diameter of 1-2 metres. The pits varied greatly in dimension, morphology and location. Most individuals were found at the base of the structure, however, some individuals had been placed in side-cavities dug into the walls. Also, some graves had been covered with large stones and some individuals had been placed on large slabs of varying morphologies and dimensions (Prats, 2013). Overall, the 27 individuals came from a total of 13 structures, including single (SJ-95, SJ-353, SJ-354, SJ-372, SJ-385), double/triple (SJ-88, SJ-354, SJ-373, SJ-385, SJ-400, SJ-86) and collective burials (SJ-86, SJ-161, SJ-296, SJ-399, SJ-418) (Table 2). The structures shown are distributed in the different areas of the excavation, although there is a greater concentration in zone 5 and 7 (Figure 9).
4.2 Final sample description

Due to poor preservation of the enamel surfaces, the final sample was reduced significantly by about 75%, down to 7 individuals (Table 3). It was composed of 2 F, 3 M, 1 M_p and lastly, 1 that could not be determined at the sex level (nd) due to the age i.e. 10-12 years old. Regarding the age groups represented there are 2 mature individuals (1 M and 1 M_p), 4 adults (2 F and 2 M) and 1 child of undetermined sex (nd) (Figure 15).

![Graphs of the final 7 individuals selected for DMTA categorised by sex and age group](image)

The selected 7 individuals used for the DMTA came from only 4 different structures (SJ-86, SJ-354, SJ-399, SJ-400). SJ-399 was a collective burial with 8 people mainly buried individually at different levels and the other 3 structures were used as double inhumations, SJ-86 and SJ-400 simultaneously and SJ-354 an individual burial. SJ-86 which had a truncated, conical form was the largest out of all silos used in this study, with a capacity of 7,066 litres. The next largest was SJ-399 (4,529 l), then SJ-400 (2,590 l) and finally SJ-354 (2,193 l) (Prats, 2013).

MIN-86-2142-1 (Figure 16) was buried slightly above and in direct contact with a 10–12-year-old who was not provided for this study. The mature, possible male was between 50-60 years old at the time of death and although parts of the skeleton were not present, the mandible was well-preserved. The mandible was intact, however, there is some damage due to
root activity and staining. Ante-mortem tooth loss (AMTL) (UrM1, UrM2, UrM3, UrPm2, LrM1, LrM3) was quite severe probably due to age and there is also very significant oblique wear and complete loss of the occlusal surface, comparable to that of MIN-400-5243-2. Perhaps due to the advanced occlusal wear there are no signs of cavities nor enamel hypoplasia. However, the individual did suffer from a periodontal disease such as Gingivitis seen from the recession of the alveolar bone (Agustí et al., 2005).

MIN-354-9085 (Figure 17) the other mature man (50-60 years old) of the sample set was buried along with a dog (Grandal-d’Anglade et al., 2019). He was buried in a flexed, lateral decubitus position against the structure wall and below the individual MIN-354-9080, a 15–17-year-old possible female. Almost all his skeleton has been preserved, however, it is in a

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3 For more details see chapter 4.3 Dental pathologies and macrowear from initial observations.

4 This female individual was so poorly preserved that only fragments of the jawbone and loose teeth were available. See Figure 33 for another similar example (i.e. MIN-296-8558).
very precarious state (Alonso, et al., 2003). His mandible has fractured in three pieces and there is extensive dental calculus and occlusal surface wear. The individual, who had extensive arthritis, also suffered from bad dental hygiene and cavities in the molars. As for many of the elderly individuals at Minferri, a large amount of dental cementum is evident due to the retraction of the alveolar bone and AMTL. It is also impossible to distinguish any signs of hypoplasia in this individual as a consequence of the large amount of calcareous concretions and calculus on the buccal surfaces and occlusal wear (Agustí, 2005).

MIN-399-5199 (Figure 18), a female, aged 25-30 at the time of death, was the last to be buried in SJ-399. Her skeleton was found in a supine position and fairly-well preserved except for the neurocranium, patellas and left hand and foot. Occlusal wear is low except in the lower right M1 (LrM1) where there is also a presence of alveolar retraction associated with
periodontal disease. There is a generally high presence of calculus, in particular in the lower jawbone and the lower right second premolar (LrPm2) has a cavity in the bone due to an alveolar abscess (Agustí, 2009). There is some damage to the enamel of the buccal surface and mandible at macro-level caused by plant root etching. Due to such poor condition of the dentition only the LrM2 could be used in the analysis.

MIN-399-5220’s (Figure 19) skeleton was completely preserved apart from some phalanges. The skeleton was crouched on his right side in a lateral cavity on the west of the silo, in a similar fashion to MIN-399-5252 who was buried in the east wall. He is described as a robust individual between 18-20 years old, whose right clavicle is more developed than his left, suggesting the repeated use of his right arm and shoulder for some kind of physical activity (Agustí, 2009). The mandible is fractured and between 10-15% of it is missing due to poor preservation. The occlusal tooth surface is very slightly worn and there is little to no wear on the M3s as they were still in the eruption process. There is no sign of cavities nor alveolar retraction, but some hypoplasia in the enamel formation.

Figure 18 - Photos of mandible from female found in silo 399 (MIN-399-5199)

MIN-399-5252 was not used for the DMTA. See Table 2 for more details.
MIN-399-5274 (Figure 20), the second female aged 30-40 at the age of death, had been placed on flat stone in a prone position with the legs bent and was the first to be buried in SJ-399. The skeleton was preserved quite well except for the left patella and foot bones and is described as robust with prominent crests for muscle attachment. The condition of her teeth shows poor dental hygiene seen by a significant alveolar abscess, AMTL of the right M2 and 4 other cavities in the molars (Agustí, 2009). This individual also shows signs of hypoplasia as is the case for many of human remains found at Minferri. Despite being intact, the mandible’s surface is fairly poorly preserved mainly due to grooves left from plant root etching on the bone and erosion. With regards to the dentition, there is significant occlusal wear and calculus, and therefore, only the left M1 on the buccal side could be included in the study. Also of note in the following analysis, the females were both represented by only one tooth each and the males by 6 in total, therefore, statistically the analysis does have much importance.
The two individuals buried in SJ-400 (MIN-400-5243-1 and MIN-400-5243-2 (Figure 21 and 22) were found in the same strata, however, some bones were disarticulated and it is presumed that this was not the primary burial site. These two individuals had the best preservation at a micro-level. The sequence of this double burial is also not known. The adult skeleton (MIN-400-5243-1, 20-30 years old) (Figure 21) was preserved well except for the tibias, fibulas, coccyx and phalanges (Agustí, 2009). It is the only mandible used in the DMTA that still has 6 molars left. At first glance the dentition appears to be in ideal condition for DMTA due to the lack of occlusal wear and generally good preservation, however, on closer inspection, hypoplasia can be seen to have affected most of the teeth. Moreover, unusual pits have formed on the enamel in both lingual and buccal sides of the molars.

Figure 21 - Photos of mandible from male found in silo 400 (MIN-400-5243-1)
The child (MIN-400-5243-2, 10-12 years old) (Figure 22) was also well preserved except the phalanges and the mandible has broken in two. The dentition is a mix of permanent and deciduous teeth. Apart from the deciduous M2s having very significant oblique gross wear and complete loss of the occlusal surface, the rest of the dental remains have little wear due to the age of the individual. There are also signs of hypoplasia in this individual, especially in the premolars and anterior teeth (Agustí, 2009). MIN-400-5243-2 is the only young individual included in the study and this must be kept in mind during the statistical analysis as the dental wear could be different in such a young person.

Figure 22 - Photos of mandible from child found in silo 400 (MIN-400-5243-2)
4.3 Dental pathologies and macrowear from initial observations

All the individuals used in the DMTA suffered from some form of dental pathology and had health-related issues with their dentition. In the mature individuals (MIN-86-2142-1; MIN-354-9085) AMTL, significant occlusal surface wear, high presence of calculus, cementum and cavities are more prolific (Figure 23). One individual, MIN-399-5252, a mature male aged <50 had lost all 6 molars and some time before death as the alveolar bone had remodelled itself filling the holes left by the tooth roots (Figure 24).

They also suffered from periodontal diseases and alveolar abscesses probably due to infections which, in some cases, affected the jaw bones as well (Figure 25). These problems were not exclusively found in adults but also occasionally appeared in children and adolescents (e.g. oblique gross wear and hypoplasia in MIN-400-5243-2). The most serious case found at Minferri was a child of 7-8 years old who had cavities in all decidual lower molars and the buccal wall of the lower left canine where there had also probably been an alveolar abscess (Agustí, 2009).

Hypoplasia, a developmental defect in enamel structure, was present in 4 out of the 7 individuals used for DMTA (Figure 26). This pathology can have both genetic and environmental causes resulting from low or premature birth weights to diabetes or viral and bacterial infections. It often suggests the occurrence of a period of ill-health or malnutrition in the first few years of the individual’s lives (Hillson, 1992). The worn-down crowns, enamel erosion, cracked and broken teeth already hint at the population having a fairly abrasive diet and/or the use of teeth as tools. Others perhaps suffered from bruxism, a condition involving excessive clenching and grinding of teeth, and MIN-400-5243-1 had impacted wisdom teeth which had not erupted correctly.

Two individuals particularly stand out as having atypical, oblique gross wear on the buccal side and occlusal surface of their M1 or M2. The complete crown is absent and less than
half of the buccal side is present. It is difficult to know exactly what this extraordinary wear could result from especially without considering the maxilla. It seems as if it could be from non-masticatory actions such as tasked-based and craft activities or even bruxism (Agustí, 2009). MIN-86-2142-1 is aged between 50-60 (Figure 16) whose left M1 and M2 was affected and MIN-400-5243-2, aged 10-12 years old (Figure 22), whose wear is a little less visibly polished, has alterations in both deciduous M2s.

Figure 23 - Photos of pathologies and dental macrowear e.g. significant occlusal surface wear, high presence of calculus, cementum and cavities (top left: MIN-372-10019; top right: MIN-354-9085; bottom left: MIN-296-8590; bottom right: MIN-399-5199)
Figure 24 - Photos of severe ante-mortem tooth loss (AMTL) in MIN-399-5252, a mature male

Figure 25 - Photos of the females studied with DMTA with probable alveolar abscesses (left: MIN-399-5199; right: MIN-399-5274)

Figure 26 - Possible hypoplasia in the individuals at Minferri (left: MIN-373-10041; right: MIN-400-5243-2)
5. Methodology

5.1 Sample selection process

There were 3 phases to the selection process which started with an initial macroscopic level elimination of some individuals due to poor preservation (Figure 27). Neither of the two individuals in SJ-88, mentioned before with the spectacular grave offerings, can be included in this study due to a high presence of calculus and post-mortem alterations of the enamel (Figure 11 & 12). Deciduous teeth were also excluded due to the differing enamel structure and range of mandibular movements. The enamel tends to be more porous and softer than in permanent teeth (Grine et al., 2005; Low et al., 2008; Machado et al., 2009) and the bite force increases throughout a child’s life (Owais et al., 2013). In past analyses, both buccal and occlusal surfaces have provided complementary information with regards to understanding the turnover of microwear patterns. (García-González et al., 2015; Hernando et al., 2020, 2021 & 2022). In this study, in order to expand the amount of diagnostic areas, all three molars, buccal and occlusal surfaces are to be included. A larger amount of data will allow for a better overview of the dental microwear in the Minferri sample and see if the results are significant and distinguishable amongst the individuals.

Figure 27 - Photos of examples of the individuals eliminated from DMTA due to poor preservation (left: lingual view of right mandible fragment of MIN-354-9085; right: lingual view of left mandible fragment MIN-88-2183)
The next phase of the selection process was using a low-magnification and a metallographic microscope (Leica DM 2500M). Thereafter, a further part of the sample was discarded due to poor enamel preservation. It was then decided not to include M3s and upper molars partly as wear could differ in different molars which has been shown to be true in studies on animals (Ramdarshan et al., 2016) For this study there were also not enough well-preserved specimens available for the numbers to be representative. Modern human teeth have different enamel, dentine (Smith et al., 2006) and cementum thicknesses which naturally must be considered throughout the analysis. Other aspects to take into account is the greater wear that the M1 tends to have (as they erupt earlier), even noticeable at a macro-level. In contrast, M3s have more variable eruption patterns and wear depending on the individual and the effect of this variability has not yet been explored (Martínez & Pérez-Pérez, 2004). Furthermore, M3s are not as common as they are prone to falling out or do not initially erupt (Petraru et al., 2020). This can be seen in the current study where originally the sample consisted of 37 M1s, 33 M2s and only 16 M3s from 27 mandibles (Table 2).

The third phase was an initial analysis with the confocal microscope and further elimination of the potential sample was necessary. It is beneficial to begin with a lower magnification to locate the studiable area and then increase to a higher level. Initially observations were made with 50x magnification, and then for the purpose of the analysis 100x and 200x were used to see if they were comparable and if one could be determined as preferable over the other. During this phase, it became apparent that many molars had alterations to the whole occlusal surface and therefore the initial intention to compare occlusal and buccal surfaces of the same tooth was not possible (Figure 28).
In DMA, buccal surfaces have often been successfully used to make dietary inferences in early hominins and modern human molars (e.g. Pérez-Pérez, et al., 2003; Romero et al., 2013; Salazar-García et al., 2016; Martínez et al., 2016). The buccal enamel surface is not affected by tooth-on-tooth contact during the chewing process and, therefore, it could be argued that it provides a clearer image of the wear patterns caused by the food consumed (Pérez-Pérez et al., 2004). It has also been argued that buccal microwear shows a longer-term dietary signature and occlusal surface, often characterised by more pits, can inform us of only the last few days of the individual's life (Romero et al., 2013; Martínez et al., 2016; Pérez-Pérez et al., 2018). Very few, if any, studies using DMTA and human buccal tooth surfaces have been undertaken except Hernando et al., 2022, and it has been proven to be a successful technique in classifying diet variations between catarrhine primate species with known dietary habits (Aliaga-Martínez et al., 2017).

The final selection was reduced by 75% to 12 teeth, 5 M1s and 7 M2s, from 7 individuals; 2 females, 3 males, 1 possible male and 1 child (Table 4). The issue of reduced samples, which applies to most archaeological studies, has also been raised by many DMA researchers and often large portions of the original material needs to be discarded due to poor preservation mainly from taphonomic processes (Teaford, 1988; Martínez & Pérez-Pérez, 2022).
2004; Ungar et al., 2008, 2012). For more details on the observations of different taphonomic effects in the Minferri sample, see results section 6.1 Taphonomic and surface alterations.

5.2 Tooth cleaning and mould elaboration

The following methodology was based on previous published works (e.g. Ungar et al., 2003, 2008; Scott et al., 2005, 2006, 2012) and under the supervision of Juan José Ibáñez at the CSIC. The first step was to clean the samples with cotton swabs soaked in 96% ethanol, in order to remove grit, dust and lipids carefully because the tooth surface can be potentially scratched. Likewise, the teeth can be cleaned with acetone to eliminate preservatives and rinsed with distilled water to remove any further residues. After the teeth were left to dry, three negative moulds were made of each molar being studied. Dental impression material Provil novo Light EN ISO 4823, type 3, light (Heraeus Kulzer GmbH, Dormagen, Germany) was applied using the dispenser (Figure 29) as recommended by various investigators (Galbany et al., 2004; Goodall et al., 2015). After being left to dry for a few minutes, the moulds were removed from the tooth and stored in dedicated properly labelled plastic bags to protect them from dust and further alteration. If necessary, they can also be cleaned with cotton swabs and ethanol.
These replicas facilitate the study of the tooth surface under the microscope and preserve a record of the individuals for future studies. They can be cut in half and placed on Blu-Tack in order to align the surface area under the lens. A device was also created by Niccolò Mazzucco, a former research scientist at the CSIC, that eases the positioning of the mould parallel with the lens (Figure 30). Positive casts were not made in this case because the teeth were available to be studied throughout and did not need to be returned to a museum for instance. Three negative impressions were made of each tooth as recommended by Bas et al. (2020). Two moulds are used to clean the tooth surface and for any particles to adhere to, allowing for the third impression to be more precise. For all teeth the third mould was used to capture the confocal scans, except one right M2 from MIN-86-2142-1 which will be used for the secondary experimental objective in this study by measuring the actual tooth surface and all three moulds for comparison.
5.3 Measurement procedure and pre-analysis protocol

One way to make any DMTA more repeatable and comparable is important to attempt to scan each tooth in a similar area. The mould or tooth surface need to be as close to horizontal as possible and always placed under the microscope in the same orientation with, for example, the distal side nearest the observer and the mesial farthest away. Also, standardising the part of the tooth which is selected for image capturing is important. Occlusal surface analyses focus on the different facets of the tooth, whereas the middle third (between the occlusal and cervical third) of the buccal surface is the most commonly used in DMA and will therefore also be applied to this research (Martínez & Pérez-Pérez, 2004; Romero et al. 2004; Galbany et al., 2004, 2006). However, it was extremely difficult to find exactly the same surface area at the same inclination and therefore the statistical results come from images with slightly different scanning positions, all of buccal surfaces. Each sample was scanned at least 5 times with the confocal microscope avoiding any taphonomic or surface alterations.

In addition to scanning the specimen in the X and Y dimensions, the focal plane can be controlled by raising and lowering the microscope stage, using a stepper motor (tiny increments of 0.1 microns). In this study, Z-scan level was increased to between 300-400 planes to capture the maximum data from the depth of the surface. The Sensofar PLu neox laser-scanning confocal model specifications used here are as follows: spatial sampling of 0.83 μm, optical resolution of 0.31 μm, vertical resolution of 20 nm and a z-step interval of 1 μm. The numerical aperture, which is important to document in a DMTA study as it can influence scanning results (Calandra et al., 2019b), at 100x magnification was 0.30 and 200x magnification was 0.50.

All scans were saved as ‘.plu’ files and treated in Digital Surf’s Premium MountainsMap© surface imaging and metrology software (version 7.4.9391). From each confocal scan, around 5 subareas of 100x100μm were manually extracted from both 100x and 200x magnification for further analysis. Prior to this, each image at 100x was 1270x950μm and
at 200x measured 636x477μm. In total, there were 60 subareas extracted for each tooth, 30 for 100x and 30 for 200x magnification. The subareas were selected where the surface was most homogenous with few or no irregularities nor signs of contamination. Another 180 subareas were collected from the individual, MIN-86-2142-1 on the tooth surface and the other two moulds.

All images were checked in 3D view for any problem areas such as the presence of abnormal peaks and valleys or missing data resulting from mould defects, poor enamel preservation or image capturing. The problem areas were edited using the “retouch” correction operator and missing data points were filled in by a smooth shape calculated from the nearest neighbouring pixels. When the surfaces were visibly contaminated or more than 5-10% of the image had to be retouched, it was not included in the study (Martisius et al., 2018; Schmidt et al., 2019). Thereafter, a template (Figure 31) was created including different correction and filter operators selected in MountainsMap© and applied to all subareas to reduce measurement noise.

Figure 31 - Screenshot of MountainsMap© window with template example
In recent studies, the filters applied in the template to the confocal scans has been shown to have an effect on the results (Arman et al., 2016; Kubo et al. 2017; Winkler & Kubo, 2022 preprint) and therefore, all steps should be clearly stated in a DMTA protocol. Since the form of the tooth is not flat, the “level” operator using the Least Squares (LS) Plane Method was applied to adjust the inclination of the surface. After that, the “remove form” operator (using a polynomial of degree 7) was added to the filter to eliminate the curvatures of the surfaces. Next, the “threshold” operator eliminates the upper and lower outliers by setting the material ratio to between 0.5 and 99.5. This reduces contamination from dust particles or moulding bubbles that are present on otherwise suitable surfaces. Finally, the non-measured points are filled in by calculating the mean surface matrix from its neighbouring valid points. The scans which had been taken directly on the tooth surface had to be inverted using the “mirror” operator in order for them to be comparable with the negative mould scans. After the template was applied to the scans, an Excel table with 104 parameters from SSFA and ISO 25178 was computed (Table 5). All the data collected is made available to others involved in future investigations to follow the general efforts towards a greater comparability and reproducibility of the DMA data.

5.4 Statistical analysis

Along with the measurement procedure protocol, the statistical analysis procedure used here has been proven to be an effective technique for DMTA (Ibáñez et al., 2020). The statistical analysis was performed using Table 5 and the software platform, IBM© SPSS© Statistics (version 28.0.1.1). A discriminant analysis (DA) was carried out to build a predictive model for group membership of discriminant functions based on combinations of predictor parameters showing different variance-covariance matrices. It was used to observe affinity between individuals and compare distributions between the classification groups i.e. females
and males; M1s and M2s; tooth surface and moulds. The classification rule of the predictive analysis is based on Bayes' theorem. Parameters which best discriminated between two or more groups were selected through a stepwise regression analysis. In all but one test, Box’s M indicated that the assumption of equality of covariance matrices was violated and therefore, in these cases a quadratic discriminant analysis (QDA) was chosen. However, this was not the case for the test between all M1s and M2s at 200x. Here, a normal discriminant analysis was applied because there was no equality in the covariance. Box's M test results indicated that the assumption of equality of covariance matrices was not violated, so the matrix of covariance within groups is used, not as separated groups as in the quadratic discriminant analysis.

The procedure was carried out to answer 5 different research questions and independent predictor parameters were automatically selected that best characterised the specific groups depending on the research question asked.

Are there differences between:

- the 7 individuals? Age groups? Macro- vs. microwear?
- females and males?
- M1s and M2s?
- tooth surface and 3 different moulds?
- 100x and 200x magnifications?

Therefore, the classification groups are as follows: all individuals (n=7); females (n=2) and males (n=3); all M1s (n=5) and M2s (n=7); M1s and M2s in only two adult males (MIN-354-9085 & MIN-399-5220). The first M1 vs. M2 test was carried out on all individuals to expand the sample size to the maximum, however, the second test reduced the variability of the analysis, i.e. two adult males both represented by an M1 and M2. Finally, the actual tooth surface and three moulds of similar buccal surfaces in one mature male, MIN-86-2142-l were compared.
Mean and standard deviation values were calculated and analysed for each significant predictor parameter in all the different tests and can be found in the supplementary materials along with structure matrix tables and a number of other tables selected from SPSS (Tables 6-11). When not already selected as a predictor parameter, the parameters $Asfc$ and $epLsar$, were also analysed and compared in each test to see if any further discrimination between groups could be made\(^6\). These two parameters were selected for this study and as mentioned in the introduction, in past research they have been considered most useful when discerning dental microwear signatures. Definitions and descriptions of the predictor parameters used for all tests can also be found in the supplementary material (Table 5). Where possible, scatter plots were produced, in which the axes represent the variance of the first two functions (Figures 48, 49 & 54-57). In the other 2 analyses i.e. females and males and all M1s and M2s, box plots were created to compare mean values of $Asfc$ and $epLsar$ (Figures 50-53).

\(^6\) See Tables 6-11 for the mean and standard deviation values of these two parameters for all 5 tests (included in the ‘Mean and St Dev’ tab)
6. Results

6.1 Taphonomic and surface alterations

Taphonomic alterations result from biological, chemical and physical processes that take place from the time of death to the fossilisation of an organism in the archaeological record or return to the lithosphere. The study of taphonomy has generally been divided into three phases in which post-mortem alterations can occur i.e. necrology, biostratinomy, and diagenesis. Thereafter, another important stage, which is not classified as taphonomy but can also alter samples, is the influence archaeologists can have on the artefact in the field or laboratory. These alterations can provide vital information not only about human and animal activity but also regarding the surrounding environment and site formation processes. It is difficult to be certain but it is believed that the burials at Minferri were covered up fairly quickly as the individuals do not display many signs of alterations from the biostratinomy phase such as disarticulation, scavenging or transportation\textsuperscript{7}. However, what is very clear is that from the initial sample of 27 individuals, 6 were so badly damaged by taphonomic processes that little or none of the mandible has been preserved and the enamel surface was destroyed rendering them impossible to be used for DMTA (Figure 32).

\textsuperscript{7} As mentioned in section 4.2 Final selection description, the two individuals in silo 400 are the exception as some parts of their skeletons were disarticulated and probably moved from their primary burial location.
At a later stage in the selection process using a low-magnification and the confocal microscope, it became apparent that some more molars had to be excluded due to the rough, irregular and abraded enamel surface (Figure 33 & 34). All the exemplary confocal scans were collected during the selection and analysis phases and are all from the buccal surface of the teeth from the Minferri individuals, unless stated otherwise. None of these scans (except Figure 35) were used in the DMTA study and are meant purely as a visual aid for the identification and description of taphonomy and surface alterations.
Figure 34 – Exemplary confocal scans of damaged enamel surface, possible post-mortem alterations such as abrasion (left: at 100x, MIN-418-5312 (M2) and right: 200x, MIN-418-5339 (M1))

Figure 35 – Exemplary confocal scans of well-preserved ante-mortem microwear texture pattern (left: at 100x, MIN-399-5199 (M2) and right: 200x, MIN-400-5243-2 (M1))

Figure 36 – Exemplary confocal scans of irregular non-ingesta related scratches or striae that are probably post-mortem (left: at 100x, MIN-161-5122 (M2) and right: 200x, MIN-400-5243-1 (M2))
As mentioned earlier and demonstrated by taphonomy experiments, alterations, although tending not to overwrite ante-mortem wear patterns, can have a significant effect on the enamel and be difficult to recognise. A combination of different agents can transform one small surface area and also vary from tooth to tooth depending on the position in the mandible. However, usually taphonomically altered surfaces can be distinguished from well-preserved enamel wear patterns at a micro-level, in particular for buccal surfaces that do not normally have pits resulting from the tooth-on-tooth crushing action (Martínez & Pérez-Pérez, 2004). Under the microscope, diagenesis processes such as chemical or physical erosion can be
identified on the enamel in differing forms ranging from scratches to prism patterning. Post-mortem scratches are often deeper, more irregular and transect the existing ante-mortem wear patterns that are present below (Figure 36 & 37). A series of parallel striae across the enamel surface is a further sign of post-mortem taphonomic processes possibly deriving from sediment abrasion (King et al., 1999; Aliaga-Martínez, 2015). Whereas on the other hand, ingesta-related wear patterns generally consist of more regular, finer striations in many different orientations and lengths (Figure 35). Erosion, normally resulting from chemical dissolution of organic tissue (Coupal & Soltysiak, 2017), softens the surface which then in turn produces chipping, rough surfaces and large pits in the enamel and bone alike (Figure 38).

Another taphonomic process which often affects skeletal remains, including the individuals from Minferri, is the dissolution of the bone cortex and tooth surface caused by probable root activity. This biotic agent along with soil fauna and microorganisms penetrate the bone and tooth structure to exploit the nutrients which can be observed at both macro- and micro-level (e.g. Figure 39 (both) & 42 (right); Figure 33 & 38 both right)). In this sample, whenever the mandible was heavily affected by taphonomic processes, generally the same was true for the dentition. Abiotic factors such as atmospheric and environmental agents are responsible for much of the surface destruction and can produce sediment or calcareous concretions or fractures in the tooth surface. For instance, the effect of hydration and rehydration can cause fissures, flaking and eventually the disintegration of surfaces (Figure 40 & 41).
Figure 39 - Photos of damage to bone and teeth from vegetation root etching and erosion (lingual view of right mandible fragment of MIN-296-8592 & left mandible fragment of MIN-296-8587)

Figure 40 – Photos of surface damage (concretions and flaking) on mandible and dentition from weathering and erosion (left: MIN-296-8592 & right: MIN-88-2145)

Figure 41 – Exemplary confocal scans of enamel defects such as cracks and pits from possible weathering and erosion (left: occlusal at 100x, MIN-400-5243-1 (M2) and right: 200x, MIN-399-5220 (M1))
Furthermore, weathering and erosion have been related to the exposure of perikymata and prisms when observing enamel wear at a microscopic level (King et al., 1999; Martínez & Pérez-Pérez, 2004). Along with oral and dental pathologies, perikymata can be an issue that especially impacts the analysis of buccal surface texture and these areas need to be avoided. Perikymata are grooves on the enamel surface formed where the striae of Retzius, slightly brownish internal growth bands, meet the surface. At 100x magnification (Figure 42, left), with a larger surface area in view, the incremental growth lines are easier to recognise, however, at 200x (Figure 42, right) it is more challenging to identify the effect they have on the enamel matrix. These grooves will surely affect the complexity and anisotropy parameter values and cannot be removed with operators in MountainsMap©. Another issue that could result from erosion, in particular acids, is the exposure of a layer of prism patterning which can often transform the whole tooth surface (Figure 43). The microstructure layer between the enamel-dentine junction (EDJ) and the outer enamel surface (OES) is made up of closely packed hydroxyapatite crystallites which form prism patterns known as Hunter-Schreger Bands (HSB). These micropatterns are believed to have evolved in different parts of the dentition in order to improve biomechanical properties and strengthen the enamel (Rensberger, 1997). The orientation and distribution of the HSB patterns in different parts of the teeth have been connected to tooth wear and enamel resistance (Lynch et al., 2010). However, the link between the prism microstructure and microwear patterns has yet to be established (Tseng, 2012). Along with the tooth structure, there is still a need for further research on the effect of enamel and dentine properties with regards to DMA (Teaford, 2007).
Figure 42 – Exemplary confocal scans of perikymata or growth lines (at 100x and 200x) (left: at 100x, MIN-399-5274 (M1) and right: 200x, MIN-399-5220 (M2))

Figure 43 – Exemplary confocal scans of prism patterns on enamel (left: at 100x, MIN-88-2142-1 (M2) and right: occlusal 200x, MIN-400-5243-1 (M3))

Figure 44 - Possible glue or varnish residue at a macro-level on mandible (MIN-400-5243-1) and confocal scan at 200x (MIN-400-5243-1 (M3))
Other alterations to the enamel surface can also be caused by excavation, preparation and conservation processes which are often unknown to the investigator carrying out the DMTA. As with many archaeological remains, these mandibles from Minferri showed signs of conservation or consolidation efforts such as glue or Paraloid residue (Figure 44) which leave quite distinctive marks but are not commonplace. Irregular or parallel striations and cracks in the enamel are frequent artefacts that often have unknown origin or cause. These alterations, as well as individual deep scratches which are differently aligned from the main texture pattern, are very likely to be post-mortem wear (Figure 45). An interesting study by Weber et al. (2021) provides comparison material including a visual guide showing common post-mortem enamel alterations. They also demonstrated that texture parameters, i.e. $\text{epLsar}$ and $\text{Vmc}$, will react differently in a variety of surfaces with known enamel alterations. Unexpectedly, in many cases parameter values of altered surfaces were not extraordinarily different to ingesta-related enamel wear patterns (Weber et al., 2021). Explanations for this could be that in most cases the enamel artefacts do not affect the whole surface and during the measurement procedure a smaller subarea of the scan is selected for analysis and a mean value is calculated after applying the pre-analysis template that removes measurement noise and outliers. These measures play an important role in the reduction of interference from abnormal, altered surface textures and anomalies.

Something not often mentioned in other DMTA publications (Mihlbacher et al., 2019; Weber et al., 2021), but was frequently observed during this study, is the issue of replication precision and moulding errors that can affect the surface textures and therefore measurement data. Small air bubbles in the silicone can be observed where the impression material has not covered the tooth surface properly (Figure 46). Scans similar to the ones found in this study

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8 See supplementary material in Weber et al., 2021
were published in the visual collection guide from Weber et al. (2021). Concretions observed on the confocal scans that resulted from unknown causes, probably ranging from post-mortem alteration, such as taphonomic artefacts to ante-mortem accumulations of dental calculus and cementum, which also greatly altered enamel surfaces (Figure 47). As mentioned before, calculus and cementum are ante-mortem alterations in the dentition, which can often be easily recognised at a macro-level and need to be avoided for DMTA (Figures 11, 12, 23 & 27). On the other hand, calcareous concretions, resulting from the precipitation of soluble salts and other types of concretions formed whilst the human remains are in direct contact with sediments and atmospheric agents, further prevent the enamel surfaces from being considered for DMTA. Overall, as can be seen from the wide diversity of taphonomic and surface alterations, careful attention must be paid whilst sampling for DMTA in order to avoid contaminated results. Furthermore, a better understanding of taphonomic effects on enamel can only lead to improved analysis sampling and therefore, more precise results.
Figure 45 – Exemplary confocal scans of the same scratches at 100x (right) and 200x (left), presumably post-mortem wear (above: MIN-399-5274 (M1) and below: MIN-400-5243-2 (M1)).

Figure 46 – Exemplary confocal scans of moulding defects in the form of bubbles or liquid on the surface (at 100x (right) and 200x (left)) (top left: MIN-86-2142-1 (M2); bottom left: MIN-354-9085 (M1); and top right: MIN-400-5243-2 (M1); bottom right: MIN-373-10041 (M2)).
6.2 DMTA statistical results

From all the test results the most commonly occurring predictor parameters were as follows: some form of Asfc i.e. Mean, HAsfc9 (n=7), Isotropy (n=7) and epLsar (n=6). This corroborates with previous studies in DMTA that mostly use Asfc and epLsar because they have shown to be most informative (e.g. Scott et al., 2005; Hernando et al., 2022). Spc, Std and Vmc all appeared 3 times, whereas the others were only present 1 or 2 times as a result of the stepwise regression analysis. All parameters and functions failing the tolerance test and/or showing non-significant discriminant capacity in the Wilks’ lambda test were removed (i.e. a p-value higher than 0.05). There were significant results for all functions of each group except for function 6 in the test between all 7 individuals and function 3 in the test for the comparison between tooth surface and moulds.

6.2.1 Comparison between all 7 individuals

The stepwise regression analysis selected the following 8 parameters to be most discriminant between the 7 groups at 100x magnification: SRC threshold, epLsar, Smc, Sdq, Sdr, Vmc, Sda, Isotropy. At 200x, the analysis selected the following 8 parameters to be most
discriminant: Reg. min scale, epLsar, Sdr, Vmc, Ssc, Sfd, Sci, Isotropy (See Table 7 for results and Table 6 for descriptions of parameters).

Figure 48 - Scatter plot of the canonical discriminant functions for all individuals at 100x magnification. However, there is one clear outlier, (1) MIN-354-9085, the mature male with occlusal macrowear who also had the highest classification results (83.3%) in his predicted group membership. He was distinguished from the rest of the groups principally by the mean value of Smc (96.71µm), which was very different from the rest. This result suggests a comparatively high complexity compared to the other Smc mean results (0.49-15.19µm). This parameter was also chosen as a predictor parameter for another test, between all M1s and M2s at 200x, and the results were similarly high (126.86µm & 64.05µm)\(^9\). Furthermore, this individual had a lower Vmc mean value (0.17µm\(^2/\mu m^3\)) compared to the others who all had an

\(^9\) See section 6.2.3 Comparison between all M1s and M2s in all individuals for more details (Table 9)
average of around double, 0.30µm²/µm³. Both mature males had lower *Vmc* and *Sdr* values than the rest of the group, however, MIN-354-9085’s values were even less than (7) MIN-86-2142-1. Having the lowest values in both parameters (*Vmc*: 0.17µm²/µm³ & *Sdr*: 0.32%) means the tooth surface is more polished, flatter, and less complex. MIN-86-2142-1 had the highest *Isotropy* mean values (54.2%).

![Figure 49 - Scatter plot of the canonical discriminant functions for all individuals at 200x (functions = 76.2% of the variance)](image)

On the other hand, at 200x there is a multivariate normal gaussian distribution between the groups (Figure 49). There are four individuals grouped together, and 3 outliers: (1) MIN-354-9085 and (7) MIN-86-2142-1, the two mature males and the child ((6) MIN-400-5243-2) all with the most important macrowear. *epLsar* was selected as a predictor parameter at both 100x and 200x magnification and overall, there was little variation in the mean values between individuals nor magnifications. All mean results fell between 0.016 and 0.018, except for the child at 100x magnification whose mean value was 0.015. The child’s mean values for *HAsfc9* were also higher than the rest of the group. This is typical from occlusal surface DMTA results in that surfaces with low anisotropy (e.g. *epLsar*) results generally also have higher complexity.
6.2.2 Comparison between males and females

The stepwise regression analysis selected the following 9 parameters to be most significant to distinguish between the two groups at 100x magnification: \( H_{Asfc9} \), \( Mean_{Asfc} \), \( StdDev_{Asfc} \), \( Mad_{Asfc} \), Fractal complexity (\( L_{sfc} \)), \( Sdq \), \( Std \), Second Direction, Third Direction. The parameters Second Direction (0.094) and \( H_{Asfc9} \) (0.852) had high p-values in the results of the test of equality and therefore they were not regarded as significant. At 200x, the following 5 parameters were considered most significant to distinguish between the two groups: \( ep_{Lsar} \), \( Ssk \), \( Sha \), \( Std \), Isotropy. (See Table 8 for results). The parameters \( ep_{Lsar} \) (0.094) and \( Sha \) (0.852) also had high p-values and therefore they were not regarded as significant at 200x.

At 100x, the mean complexity \( L_{sfc} \) parameter values between females (5.82%) and males (2.65%) varied significantly, females showing a higher roughness or surface relief.

Expectedly, the other complexity parameters (\( H_{Asfc8} \), \( Median_{Asfc} \), \( Mean_{Asfc} \), \( StdDev_{Asfc} \) &
MadAsfc) also all had higher mean values for females. However, as can be seen in Figure 50, females have a slightly lower value in $H_{Asfc9}$. This parameter despite being a commonly used parameter, was regarded as not statistically significant in the test of equality.

At 200x, all the complexity parameter results were also consistently higher in females. With regards to anisotropy parameter results at 100x magnification, $epl_{sar}$ mean values were slightly higher in females (0.01698) than males (0.01642) and at 200x females slightly lower (0.01685) than males (0.017182) (Figure 50 & 51). However at 200x $epl_{sar}$ had a high p-value and therefore, is not statistically significant. The parameter $Std$ showed substantial variation between males and females at 100x (F: -0.76° & M: -19.40°).
6.2.3 Comparison between all M1s and M2s in all individuals

The stepwise regression analysis selected the following 3 parameters to be most significant to distinguish between the 2 groups at 100x magnification: Smooth-rough crossover (SRC), Svi, Isotropy. At 200x, the following 7 parameters were selected: MedianAsfc, Smooth-rough crossover (SRC), Reg. min scale, Reg. coefficient R², Smc, Svk, Sds. However, SRC was not regarded as significant as it had a p-value of 0.242 and therefore was not used (See Table 9 for results).

In Table 9 the mean values of complexity parameters and epLsar at 100x magnification are all higher in M1s than M2s. Isotropy is however lower in M1s at 100x (M1:41.17 & M2: 47.00) At 200x, this is not the case, when the same parameters were compared. Contradictorily M1s had lower results, except HAsfc9 which, curiously, had slightly higher results in M1s. However, in general a distinction in the average mean value parameter results between M1 and M2 can be made. For example, Smc at 200x shows a large variation between M1s and M2s (M1:126.86 & M2: 64.05).

![Figure 52 - Box plots of all values of HAsfc9 and epLsar with M1s and M2s at 100x magnification](image-url)
From Figure 52 & 53, it can be seen that from all the measurements of $H_{Asfc9}$ and $ep_{Lsar}$ there is not a large difference in values. At 100x magnification the distinctions between the two molars can be more easily observed than at 200x. The results in the box plots further support that overall the M1s have slightly higher values in complexity parameters.
6.2.4 Comparison between M1 and M2 of two different individuals

The stepwise regression analysis selected the following 6 parameters to be most significant to distinguish between the 4 groups at 100x magnification: MeanAsfc, epLsar, Spc, Spk, Isotropy, First Direction. At 200x, the analysis selected the following 10 parameters: MeanAsfc, Reg. min scale, Reg. max scale, Reg. coefficient $R^2$, epLsar, Sal, Vmc, Spc, Smr2, Isotropy. Reg. max scale and Smr2 both had high p-values (0.131 and 0.936) (See Table 10 for results).

![Canonical Discriminant Functions](image)

Figure 54 - Scatter plot of the canonical discriminant functions for 2 individuals with both M1 and M2 at 100x (functions = 95.2% of the variance)

At 100x, the scatter plot (Figure 54) similar distances between individuals and teeth can be seen but the distribution is almost random. Spk mean values varied greatly between the two individuals as MIN-354-9085 (mature male) had much higher values (M1: 36.13µm & M2: 81.25µm) than MIN-399-5220 (young adult male) (M1: 0.33µm & M2: 0.29µm). MeanAsfc also varied greatly between individuals and teeth. M1s had higher mean values in both individuals and overall, the young male had much higher MeanAsfc. Also of note, the M1s had
a distinctively higher First Direction value: 83.99° & 95.20° than the M2s with 69.48° & 60.29°.

Figure 55 - Scatter plot of the canonical discriminant functions for 2 individuals with both M1 and M2 at 200x magnifications (functions = 90.4% of the variance)

Here at 200x in Figure 55, there is a slight parabolic distribution that can be observed and similar distances between the individuals and teeth. In Figures 54 and 55, it can be seen that 200x magnifications was more effective for classifying the different teeth in the two individuals. MeanAsfc was selected as a predictor parameter for both magnifications, however, at 200x, there was not such a clear distinction between individuals and teeth as the values were more similar. However, MIN-399-5220 (young adult male) still had slightly higher values than MIN-354-9085.
6.2.5 Comparison between tooth surface and 3 different moulds

The stepwise regression analysis selected the following 6 parameters to be most significant to distinguish between the 4 groups at 100x magnification: Sk, Str, Spd, Spc, Stdi, St. At 200x, the following 5 parameters were selected: epLsar, Std, Vm, Svi, Isotropy. (See Table 11 for results).

![Canonical discriminant functions](image)

Figure 56 - Scatter plot of the canonical discriminant functions comparing x3 moulds with original tooth surface in MIN-86-2142-1 at 100x (functions = 94.8% of the variance)

At 100x in Figure 56, differences can be clearly seen between moulds and tooth surface. Remarkably, the tooth surface measurements represented in the scatter plot are closest and therefore most similar to mould 3. Whilst comparing mean values for all four groups, Sk and Str were the most similar in the tooth and third mould (Table 11). Sk is related to the core roughness depth and Str an anisotropy parameter measuring the orientation and length of the features. The results for both parameters progressively decreased in value from the first mould with the highest mean, then the second, third and finally the tooth. Having lower results in these
parameters means that the levels of roughness, complexity and variation of the orientation and length of features on the tooth surface are slightly reduced.

However, using 200x magnification, it can be seen in Figure 57 that the hypothesis of mould 3 being more accurate is not confirmed. In this case, mould 1 is actually closer to the tooth surface on the scatter plot and the 3 moulds are more strongly grouped together. The tooth surface measurements are distinctly separated. Here, Std and Isotropy show the most variation in the mean values between the 4 groups. The tooth surface had significantly lower values (Std: 75.93° and Isotropy: 30.76%) than the rest of the group. Both these parameters represent the orientation and direction of the texture wear patterns.

In summary, the classification results from the stepwise regression analysis were higher at 200x magnification in three tests i.e. between all the individuals and twice when comparing M1s vs. M2s. On the other hand, when comparing females and males (f/m: 82.5% vs. 71.7%) and the experiment with the tooth surface and three moulds, both the classification
results were better in 100x. In the test of the tooth surface and moulds at 100x, where the hypothesis was confirmed, the classification results were significantly higher compared to the test at 200x (80.8% vs. 64.2%).
7. Discussion

7.1 DMTA results on the anthropological remains from Minferri

The creation of a protocol in order to account for the variation in DMTA with archaeological human dental remains is complex due to the extensive potential for observer errors and number of possible modifications to the technique. Therefore it is paramount that further experimentation, testing and standardisation of some aspects take precedence in future studies (Weber et al., 2021; Winkler & Kubo, 2022). Within this study, an assessment of some of the factors that can influence the results has been made through carrying out a protocol and statistical analysis with the ensemble from Minferri. The focus was primarily methodological by examining the mould comparability and magnifications. Also, anthropological and archaeological data on the variation between demographics i.e. sex, age and molar type, burial typology were included, in order to be able to infer social aspects in the interpretation of the analysed ensembles. Due to preservation issues and tooth surface alterations, only 25% of the sample was considered suitable for DMTA. A further issue, that is particular to studying buccal tooth surfaces, is the perikymata that often transform a large part of the enamel texture rendering it impossible to study. The results of the analysis of the taphonomic and superficial alterations that affected the mandibles have been systematised in section 6.1. A selection of images shows the severity and diversity of the agents and phenomena that affected the archaeological sample (Figures 23-28, 32-34 & 36-47). As a result, the number of individuals featured in each of the tests is low and therefore the statistics need to be interpreted with caution, pending future studies that will provide more samples and results that will complement and expand the vision of this study.

The diverse behaviour and cultures within human populations add further complications and variety in tooth wear resulting from, for example, food processing or using the mandible as a “third hand”. Additionally, seasonality of diets has been shown to have an
influence on wear patterns due to the impermanent nature of dental microwear (i.e. “the Last Supper” effect) (Merceron, et al., 2010; Karriger, et al., 2016; Pérez-Pérez, et al., 2018). If the results from the recent study with rats are comparable with humans, around 2 weeks to a month is needed for the surface to overwrite itself (Winkler et al., 2020). For many species, as is the case for the humans from Minferri, it is difficult to be certain of the seasonal variation in their diets and how this would be captured in the wear caused during the individual’s last moments of life. On the other hand, whilst carrying out a DMTA protocol, many other aspects from a technical or methodological perspective can vary. The initial sample selection phase along with the mould elaboration, image capturing with the confocal, pre-analysis treatment in metrology software and the statistical analysis can all influence the results. These factors need to be taken into consideration in any rigorous study in this field (e.g. Arman, et al., 2016; Kubo, et al., 2017; Macdonald, et al., 2018; Calandra, et al., 2019) (Figure 58).

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Figure 58 - Potential drivers of intraspecific DMTA variation (adapted from source: Arman et al., 2019)
More standardisation, communication and investigation into these variations should lead to more reliable and comparable DMTA results for inter-laboratory research.

The replicability and precision of mould and cast impression material is a part of the protocol that has been questioned in past DMA research. In order to explore this issue a preliminary test was carried out to compare texture parameter values between the actual tooth surface of MIN-86-2142-1 and 3 different moulds made of the same area. The hypothesis was that the results from the third mould should be more similar to the values from the original tooth surface as suggested by Bas et al. (2020). At 100x magnification, the hypothesis appears to be proven correct and the variation between groups was apparent in parameters $Sk^{10}$ and $Str^{11}$. However, at 200x this was not the case and in fact mould 1 was closer to the tooth surface than the other moulds. Importantly, at both magnifications the 3 moulds were distinct from the tooth surface and at 200x they can be seen quite closely grouped together (Figures 56 & 57). Overall, the mean values in complexity and anisotropy were generally higher in the 3 moulds which suggests more roughness and less uniformity in the microwear patterns. This could result from the teeth not being sufficiently clean and therefore, the replicas have additional concretions adhered to the surface. This hypothesis would need additional experimentation to be able to be confirmed. It could be argued that at 200x magnification the mould impression material is not viscous enough to provide the precision needed at such a high magnification. Furthermore, perhaps to represent the complexity of the surface texture, the subarea extracted (100x100μm) would need to be enlarged. Differences could also have resulted from the issue of not having captured and extracted exactly the same surface area and features. These observer-induced errors could therefore lead to intra- and inter-surface variations rendering

$Sk$: height parameter measuring the core material parameter height (core roughness depth)

$Str$: spacial parameter measuring surface texture aspect ratio (uniformity or isotropy of surface)
their comparison difficult. Future studies will need to take into account these variations for further comparisons between the tooth surface and moulds to be made.

In DMTA the standard magnification is 100x and there is very little published work on the influence that different microscope magnifications have on enamel surface analyses (Mihlbachler & Beatty, 2012; Kubo et al., 2017). 100x magnification is more commonly used, and from first-hand experience, this is probably due to the diagnostic area being larger and the wear patterns more readily identifiable. Preliminary comparisons between 100x and 200x magnifications were made to assess the precision of each and whether one could be preferential over the other. During the analysis the surface features and areas captured were not exactly the same which could have contributed to the variation in the results. Again, the issue could also be associated with the subarea extraction size, in that the same area i.e. 100x100μm was used throughout the whole study. If the subarea size extracted had been doubled at 200x magnification, then results would have been more comparable to those at 100x. The same could be said for taking a smaller subarea i.e. 50x50μm from images at 100x. The influence that different levels of magnification and sizes of subareas has on DMTA results requires further investigation. To avoid issues of intra- and inter-surface variability during the measurement or sampling procedure, exactly the same features or surface area should be used in future investigations. During the image capturing process with the confocal, screenshots of specific features can be made to facilitate relocating the same surface area and orientation for the following sample that is scanned (Arman et al., 2016).

It has been shown in samples from sheep and carnivores that there is variation in dental wear depending on the tooth position i.e. upper or lower (Ramdarshan et al., 2017) and M1 or M2 (Tanis et al., 2018). However, to date, there has been a lack of published work making comparisons between M1s and M2s or, in fact, upper and lower human dentition with DMTA.
It is difficult to make any interpretations from the two tests carried out due to low numbers and a lack of comparability in the sample (i.e. mix of left and right molars and variability between individuals). The reason for including all molars in one test was an attempt to increase an already small sample, however, the results remained insignificant. Later, in the fourth test, which included two male individuals (MIN-354-9085 & MIN-399-5220) who were both represented by an M1 and M2, a more refined comparison between molars was made. This involved less variability between the specimens (i.e. same number and type of tooth and tooth surface from two adult males). In both tests there were differences between M1s and M2s and between individuals (Figures 54 & 55). This could lead to the hypothesis that there are differences between different molar types, however, it can be seen from the test that inter-population variation is of greater significance. The variation between left and right sides of the mandible could have affected these results and should also be taken into consideration in further analysis. The different parameter results could also have come from the age difference (18-20yrs. vs. 50-60yrs). The M1s have a distinctive higher First Direction parameter value at 100x (M1: 83.99° & 95.20° & M2: 69.48° & 60.29°), however, these differences probably resulted from the fact that all the moulds might not have had exactly the same orientation and positioning under the confocal lens.

A further area of interest was whether wear texture patterns differ in females and males to be able to explore sexual inequalities in diet. Excluding the possible male (MIN-86-2142-1) and the child (MIN-400-5243-2) from the statistical test the 3 males (1 mature and 2 adults) and 2 adult females were separated into two groups. The mean parameter values showed significant differences between sexes (see section 6.2.2) at both 100x and 200x. For example, the complexity parameter, Lsfc, indicates females have a higher surface roughness or relief. In general, females had much higher mean values in the complexity parameters than males and unusually high in comparison to Hernando and team’s average results for buccal molar surfaces.
(Table 2, Hernando et al., 2022). This complex microwear texture has high levels of pitting and intricacy, which tends to result from a harder, more brittle diet, perhaps with large amounts of dust and grit from food processing, for example. These high values could also suggest that taphonomic alterations may have influenced the complexity of the microwear patterns. On the other hand, females have lower $epLsar$ values than males which tends to be a common connection between results with higher $Asfc$ parameter values in DMTA. As mentioned earlier, if the tooth surface has a higher $Asfc$ value it also tends to have a lower value $epLsar^{12}$.

Males and females also had contradictory classification results from stepwise regression analysis regarding the two magnifications. At 100x magnification, the group of males was more classifiable according to the stepwise analysis (M:89.4% & F:61.7%) and at 200x, females were more distinctively grouped compared to males (M:68.9% & F:80%). At 100x, the overall classification result was higher with 82.5% of the original groups correctly classified. Furthermore, the results from 100x magnification are more coherent with those provided by the isotope analysis, in that males have more uniformity in their results and females show a greater dispersion. Overall, it could be hypothesised that the individuals at Minferri had a fairly abrasive diet and females perhaps even slightly more. A possible hypothesis is that females supplemented their diets with more ground cereals which could not only account for the high level of wear but also the poor dental health. Oral bacteria feeding on the glucose in flour produces additional acids which eventually cause tooth decay. Comparing the 4 adults in this study, the 2 females had more cavities and dental pathologies than the males (Table 3). Considering that meat has proven to leave little trace on enamel, it could be speculated that males consumed this more regularly than women and therefore had less complexity to their

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12 See 6.2.1 Comparison between all 7 individuals for reference to this
enamel microwear texture. However, further interpretations go beyond the methodological approach of this study and require more extensive investigation with larger samples.

The results provided by DMTA are consistent with those from a larger study on the human diet at Minferri through $\delta^{15}$N and $\delta^{13}$C isotopic analysis (Grandal et al. 2019). The sample comprises a total of 37 individuals (8 adult males, 14 adult females and 15 sex-indeterminate remains (9 infants, 2 young adults and 2 adults). The isotopic results suggest differences amongst the population when it comes to their diets and distinctions between females and males can be made. More specifically, males presented a more protein-rich and homogenous diet within the group. In contrast, females showed much more diversification, some a higher protein intake (with ranges comparable to males) and others more vegetarian diets associated with higher cereal consumption (Grandal-d’Anglade et al., 2019). As a consequence, the DMTA results are promising as they have allowed us to identify and confirm the diversity and differences between existing diets and sexes in part of Minferri’s population. This opens new perspectives of applicability in other anthropological assemblages and gives more consistency to the hypotheses advocating sexual inequalities in Bronze Age societies visible through the study of human diet (e.g. Varalli et al., 2016; Kousta, 2017).

To date only limited DMTA research has been carried out into categorising dietary signatures with regards to human sexual dimorphism and some have suggested that there is no or little difference between sexes (Schmidt et al., 2015, 2016; Hernando et al., 2021). On the other hand, an in vivo analysis of Hadza foragers in Tanzania showed that there was a variation in the dispersion of parameter values between sexes in the dry and wet seasons (Ungar et al., 2019). This suggests that the females and males had differing diets depending on the season which could potentially relate to the population of Minferri. Furthermore, macrowear and SEM analyses have shown that Bronze Age females from the Iberian Peninsula were often more likely to have abnormal macrowear in comparison to males, possibly related to task-based
and craft activities (Fidalgo et al., 2020; Lozano et al., 2021). Smith and team using cross-sectioned human upper and lower molars calculated that average enamel measurements were thicker in females and differed amongst teeth (Smith et al., 2006).

Other aspects that can vary greatly when studying humans are not only demographics such as age and sex, but also masticatory patterns (Youssef et al., 1997) and condition of the dentition and mandible (Mahoney, 2006). For example, if an individual has some form of pathology leading to pain on one side whilst eating, it logically follows that they would start to favour the other side of the mouth. Were someone right-handed, might it imply that they are more likely to use the right side of the mouth for chewing or as a third hand whilst crafting?

As has been shown in the results from 200x magnification between all the individuals there is a tentative connection between macro- and microwear. It suggests that the individuals who had large amounts of macrowear (MIN-86-2142-1, MIN-354-9085 & MIN-400-5243-2) appeared as outliers in the scatter plots (Figures 48 & 49) and had higher complexity values. In general, throughout the sample it can be seen at a macro-level that as the individuals get older there is an increased level of wear, pathologies and AMTL. Furthermore, the two mature adults and the two females all suffered from alveolar retraction, abscesses and cavities whereas the other 2 adults and child did not. From the statistics, it cannot be clearly seen if there is any relation between microwear and age or condition of dentition which may be due to the small sample. Once again, the sample was too small to make any conclusions about these matters and further investigation is required in order to draw any conclusions.

7.2 Comparison of Minferri results with other studies

It is difficult to make comparisons with other statistical results from the field due to there being very little comparative research using the same variables (i.e. human buccal molar surface texture analysis with confocal microscopy). As has been found in various other works
with ancient humans or animals there is a difference between occlusal and buccal surfaces (e.g. Scott et al., 2005, 2012; Aliaga-Martínez, et al., 2017). Buccal wear signatures reflect a longer time frame (Jarošová, 2008), however, occlusal dental microwear is ephemeral and the turn-over rate is in the range of just a few weeks (Teaford & Oyen 1989; Teaford, 1994). That said, the mean values of complexity parameter results from this study (Tables 7-11) appear high in comparison to other DMTA studies in general. The only other paper that has published DMTA results with parameter mean values on human buccal tooth surface has quite different values than this study (Hernando et al., 2022). In this analysis, epLsar results were always between 0.016 and 0.018 and this parameter was chosen as being statistically significant in the stepwise regression analysis. However, due to changes made by Digital Surf to the parameters in MountainsMap© the results for epLsar are similar except for one decimal position less. That is to say, in past studies using older and different versions of metrology software, the value for epLsar would normally be closer to 0.0016. This issue further emphasises the variation that can be found in DMTA results. Moreover, the MountainsMap© version used in this analysis selected the following complexity parameters: HAsfc9, HAsfc81, MedianAsfc, MeanAsfc, StdDevAsfc, MadAsfc, Fractal complexity (Lsfc) and the standard Asfc was not automatically included. It begs the question as to how much other variation is due to the version or brand of software that is chosen to measure and analyse the confocal images. Moreover, is standardisation really a possibility whilst a variety of protocols are being applied using a number of confocal models and metrology software, and if so, to what extent? Studies have already shown that there is microscope intervariability and also, depending on which filters are applied during the pre-analysis treatment procedure, there can be variations in the results (Arman et al., 2016; Kubo et al., 2017; Winkler & Kubo, 2022 preprint). While some of the steps in the protocol are completely automated and reproducible, the acquisition of data is not completely free from human variation and error. The correct selection and treatment of the
tooth scans requires experience, know-how and precision. Once again, many different aspects can influence a study and therefore these technical specifications and settings throughout the measurement and sampling procedure should be clearly stated in any DMTA report.

7.3 Future perspectives for the application of DMTA in anthropological assemblages

Future work should involve the expansion of the sample size, possibly with the same protocol but a different type of tooth i.e. canines or third molars. The results could also be compared with other contemporary archaeological sites, such as the Cantorella (Maldà, Urgell) (Abad et al., 2018), Can Roqueta (Sabadell, Vallès Occidental) (Palomo et al., 2016) and/or Mas d’En Boixos (Pacs del Penedès, Alt Penedès) (Bouso et al., 2004). In the future, blind testing could be carried out in various stages of the DMTA protocol to reduce the possibility of observer bias or error (Evans, 2014). This could assist in standardising the various steps of the technique i.e. subarea extraction or pre-analysis treatment process. Also, stepwise regression analysis would not be used in the future. The rationale being that DMA researchers largely apply a pairwise comparison analysis and that, secondly, this type of statistical analysis has shown to lead to biases and to be ineffective especially when using large amounts of parameters (Thompson, 1995; Smith, 2018).

Additional comparisons between moulds relative to a tooth surface using exactly the same subareas and features on all scans could be carried out. Future research into the effect of different-sized subarea extractions and magnifications could lead to a better understanding of dental microwear surface textures. These parts of the protocol along with the pre-analysis treatment and applied filters could be steps which are eventually standardised. Ramdarshan et al. (2017) has already noted that “a 200x200μm surface allows for better differentiation between dietary categories, as opposed to analysing smaller surfaces” and that standardisation is a higher priority than enlarging sample sizes. Furthermore, Arman and team recommended
using a softer filter treatment in order to reduce variability between microscopes (Arman et al., 2016). Winkler and Kubo have also proposed a “roadmap” for the pre-analysis protocol including the most stable parameters to use\textsuperscript{13} and those to avoid (nMotif and Spd). (Kubo et al., 2017; Winkler & Kubo, 2022 preprint). Refining and standardising the protocol will allow for datasets from different studies to be comparable and will reduce the variation between inter-laboratory research.

A wider knowledge is needed on the consequences of amalgamations of food items, macro-/microwear and ante- or post-mortem alterations, especially in human buccal surfaces. Additional experimentation using DMTA and the human dentition is required, particularly with regards to studying variations of in vivo humans, in line with Correia and team (2020). Moreover, a better understanding of different microwear patterns and how they are expressed in the wide variety of surface texture parameters is needed. As can be seen from the aforementioned, the diversity in DMTA is extensive which can lead to issues and limitations within the technique. Not only can these problems arise from the initial capture of the confocal scan due to taphonomy, mould defects and/or the alignment under the lens, but also in the measurement procedure and surface texture parameter values. Standardisation in some aspects of the protocol will reduce a level of variability in order to allow for inter-laboratory comparisons of DMTA results. This paper lays the foundations for future investigations and provides a selection of suggestions for improving and standardising parts of a DMTA protocol for human archaeological remains.

\textsuperscript{13} “The height parameters: S10z, Sku, Sp, meh and metf did not show significant differences between microscopes regardless of the filter routine.” (Winkler & Kubo, 2022 preprint)
8. Conclusions

In order to study the subsistence strategies of past populations, a holistic approach is needed, which allows for the interweaving of data from different methodologies and lines of archaeological evidence. This approach is the one we wanted to work with in this paper, as we have examined the DMTA signatures of early Bronze Age population at the Minferri site and contrasted them with the data provided by anthropological (age, sex and pathology) and diet isotopic analyses.

In this paper, the potential and limitations of DMTA for the analysis of Bronze Age human dental wear have been explored by establishing a protocol and carrying out preliminary testing on buccal molar surfaces. Despite the reduced sample size, the results obtained are promising, as they have shown some significant differences within the population of Minferri. From an anthropological perspective, variation in parameter values between sexes has been observed and therefore perhaps also their diet and gender roles within the community. Moreover, the results between males and females were consistent with the oral pathologies and diet isotopic analysis in that females showed more diversification in both isotope and dental wear signatures at 100x. The DMTA reinforces the hypothesis that the Minferri women would have a more vegetarian and abrasive diet due to a higher consumption of cereals, compared to the more protein-rich diet of the men. Tentative conclusions could also be drawn in that the differences between age groups and position of tooth are reflected in the dental micro- and macrowear.

From a methodological angle, this research demonstrates that there are slight differences between the moulds when compared to the same tooth surface and also between magnifications. Additionally, some of the impact that taphonomic and surface alterations can have on archaeological samples has been portrayed. Being a preliminary study, this work
provides a basis for wider-reaching, future investigations with larger sample sizes. It also raises many questions and challenges for the future that still need to be addressed in more extensive studies.

To be able to understand the diet of modern through their diet is of significant scientific interest. However, the technique is still plagued with uncertainties and even preferred protocols are still under debate and re-evaluation. Indeed, much experimentation and collaborative investigations remain to be done. By way of an example, it remains unclear whether most microwear traces can be attributed, with any degree of certainty to any one factor i.e. feeding, tool use, parafunctional habits, pathologies, taphonomy, storage, handling or procedural treatments. More archaeological experimentation with human dentition is needed to improve DMTA interpretations and even more so in buccal molar surfaces. It is considered that this study could provide the basis for further DMTA researchers through developing on aspects and issues discussed within the report. This should lead to an enhanced consistency and confidence in this area of research.
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Scatter plot of the canonical discriminant functions comparing x3 moulds with original tooth surface in MIN-86-2142-1 at 100x (functions = 94.8% of the variance)

Scatter plot of the canonical discriminant functions for 2 individuals with both M1 and M2 at 200x (functions = 95.2% of the variance)

Scatter plot of the canonical discriminant functions for 2 individuals with both M1 and M2 at 100x (functions = 90.4% of the variance)

Scatter plot of the canonical discriminant functions comparing x3 moulds with original tooth surface in MIN-86-2142-1 at 100x (functions = 67.6% of the variance)
Scatter plot of the canonical discriminant functions comparing x3 moulds with original tooth surface in MIN-86-2142-1 at 200x (functions = 99.2% of the variance)

Potential drivers of intraspecific DMTA variation (adapted from source: Arman et al., 2019)

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